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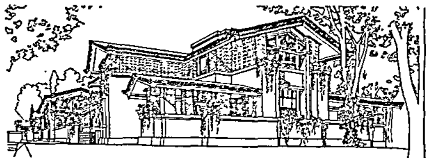
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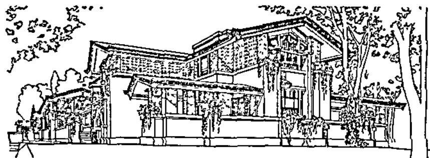
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FOREWORD

OUR LIVING CHEMISTRY SERIES was conceived by Editor and Publisher to advance our newer knowledge of chemical medicine in the cause of clinical practice. The interdependence of chemistry and medicine is so great that physicians are turning to chemistry, and chemists to medicine in order to understand the underlying basis of life processes in health and disease. Once chemical truths, proofs and convictions become sound foundations for clinical phenomena, key hybrid investigators clarify the bewildering panorama of biochemical progress for application in everyday practice, stimulation of experimental research and extension of postgraduate instruction. Each of our monographs thus unravels the chemical mechanisms and clinical management of many diseases that have remained relatively static in the minds of medical men for Three Thousand Years. Our new Series is charged with the nisus élan of chemical wisdom, supreme in choice of international authors, optimal in standards of chemical scholarship, provocative in imagination for experimental research, comprehensive in discussions of scientific medicine, and authoritative in chemical perspectives of human disorders.

Dr. Allison of New Brunswick presents a complete comprehensive interpretation of the biochemistry of food proteins, correlating complex structure with physiological function. The newer microchemical methods must unravel nutritional dynamics in human as well as in animal subjects under all physiological and pathological conditions. The concept of dietary proteins must be extended to include nitrogen-containing compounds beyond amino acids. The practice of separating dietary proteins from other nutrients must be abandoned in the light of the newer knowledge of the metabolic pool. The notion of protein utilization as a function of dietary amino-acid patterns must include nitrogen and caloric intake as well as the metabolic status of the individual. The protein value of foods must be reexamined in terms of the individual variations in biocatalysts and the increasing number of pharmacological

agents affecting human metabolism. Dr. Allison reaches formidable conclusions from his scintilating discussions of dietary proteins in health and disease, revealing many truths that have not yet reached clinical texts.

I. NEWTON KUGELMASS, M.D., PH.D., SC.D., *Editor*

CONTENTS

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INTRODUCTION

Proteins form the "warp and the woof" of all protoplasm, the cellular proteins being specific in structure for each type of living system. Proteins also are the enzymes with catalytically active surfaces which often include non-protein units such as vitamins. Proteins, combined with non-protein units, are said to be conjugated and as such they take part in many ways in the structure and metabolism of the living system. Proteins combined with lipids, for example, form lipoproteins which are essential features of the internal and external cellular membranes and interfaces. Combined with nucleic acids, proteins become nucleoproteins some of which are centered in the nucleus of cells and contain the genes of inheritance. Other nucleoproteins, organized in the cytoplasm, may be the templates for cellular protein synthesis. Proteins also form the antibodies to disease. The following discussion of proteins in health and disease, therefore, will emphasize the chemical dynamics of these structural protein units. The discussion will be centered around the importance of dietary amino acids as the building blocks of cellular proteins and will be introduced by a brief review of the following basic concepts: (a) indispensable amino acids (b) the dynamic state (c) amino acid transport (d) protein synthesis (e) catabolism vs. anabolism (f) labile protein reserves (g) protein malnutrition (h) resistance to disease (i) specific amino acid deficiencies.

INTRODUCTION

PROTEINS FORM the "warp and the woof" of all protoplasm, the cellular proteins being specific in structure for each type of living system. Proteins also are the enzymes with catalytically active surfaces which often include non-protein units such as vitamins. Proteins, combined with non-protein units, are said to be conjugated and as such they take part in many ways in the structure and metabolism of the living system. Proteins combined with lipids, for example, form lipoproteins which are essential features of the internal and external cellular membranes and interfaces. Combined with nucleic acids, proteins become nucleoproteins some of which are centered in the nucleus of cells and contain the genes of inheritance. Other nucleoproteins, organized in the cytoplasm, may be the templates for cellular protein synthesis. Proteins also form the antibodies to disease. The following discussion of proteins in health and disease, therefore, will emphasize the chemical dynamics of these structural protein units. The discussion will be centered around the importance of dietary amino acids as the building blocks of cellular proteins and will be introduced by a brief review of the following basic concepts: (a) indispensable amino acids (b) the dynamic state (c) amino acid transport (d) protein synthesis (e) catabolism vs. anabolism (f) labile protein reserves (g) protein malnutrition (h) resistance to disease (i) specific amino acid deficiencies.

of the first eight amino acids, listed above, for maintenance. Emphasis in this monograph will be placed upon these eight but as the data are presented it will become increasingly evident that the relative amounts and proportions of the dispensable amino acids can also effect the rate of cellular protein synthesis (9).

THE DYNAMIC STATE

The concept of a dynamic state of some of the cellular proteins was emphasized by Whipple and his colleagues (10) who explained their data on plasma protein regeneration by visualizing an "ebb and flow" of amino acids, possibly through many pathways, from one tissue protein to another. Schoenheimer, Rittenberg and associates developed further evidence for this concept of a dynamic state by showing an interchange of N^{15} tagged nitrogen from one tissue protein to another (11, 12). Thus the concept grew that some tissue proteins are continually being broken down and resynthesized thereby contributing to and taking from a metabolic pool of amino acids. The assumption was made that the dietary amino acids are absorbed into the body to become a part of this pool. Each intracellular pool could be considered as a member of a larger metabolic source, integrated through intra and extracellular fluids. The amino acids in these cellular pools would come from both dietary and tissue sources. Every cell may not be capable of synthesizing all of the dispensable amino acids, thereby needing more than the eight indispensable acids from extra cellular sources (13).

AMINO ACID TRANSPORT

Such a dynamic state emphasizes the importance of transportation of amino acids into and out of cells. Christensen (14) has directed attention to the relation between the ability of a cell to take up amino acids and its ability to synthesize cellular proteins. Riggs, *et al.* (15) has presented an interesting scheme for explaining cellular uptake of amino acids which involves a carrier and an active role of sodium and potassium ions in this interchange across cell membranes. Munro (16) also suggested that amino acids are sequestered in muscle cells when insulin is brought forth during the influx of carbohydrate. Following this phase of carbo-

BASIC CONCEPTS

THE INDISPENSABLE AMINO ACIDS

DIETARY PROTEINS are hydrolyzed through digestion in the gastrointestinal tract into amino acids which are absorbed into the body to become building blocks for protein synthesis. Some twenty to twenty-two different amino acids are required for this synthesis of cellular proteins. Most of these acids can be synthesized in the body of animals if sufficient amino acid nitrogen is supplied by the dietary protein. Some of the amino acids, however, cannot be synthesized in adequate amounts to meet the needs for metabolism so that they must be supplied *in situ* by the diet. Such acids are called "essential" or "indispensable" and their significance in nutrition was illustrated by the classical experiments of Rose and associates (1). These experiments demonstrated that the primary functions of dietary proteins are: (a) to furnish the indispensable amino acids in proper amounts and proportions for protein synthesis, and (b) to supply additional nitrogen in a form for synthesis of the dispensable amino acids and other nitrogenous constituents of tissues.

The data in the literature suggest that man and animal species except ruminants require a continuous dietary supply of the amino acids, leucine, isoleucine, lysine, phenylalanine, methionine, threonine, tryptophan and valine (1-7). Histidine is needed also for growth of rats and dogs but not for maintenance in adult man. This lack of ability of some animal tissues to synthesize the above nine amino acids was demonstrated by Steele (8) who utilized radioactive carbon to show that carbohydrate could not supply any carbon for the synthesis of these nine, except for the methyl group in methionine. On the other hand, carbohydrate furnished carbon for the synthesis of the dispensable amino acids. The amino acid arginine, is also indispensable in the diet of birds and may be necessary in the diet of certain other animals when the rate of synthesis of arginine is inadequate to meet metabolic needs. However, on the basis of present evidence, man requires a dietary source

has been developed recently by a number of workers (19-24). The S-RNA appears to be a protein-free ribonucleic acid with a molecular weight of only 20,000 to 30,000. The amino acids from the metabolic pool are thought to be accepted by S-RNA after they have been activated (21), and there may be a separate species of S-RNA for each amino acid (24).

The amino acids bound to S-RNA are transferred to ribonucleoprotein (RNA) of cellular particulate fractions such as the microsomes, this transfer taking place if each amino acid needed for synthesis is present and bound to its own S-RNA. The microsomal RNA is combined with protein, has a molecular weight of about 2,000,000, and is found in all cells capable of synthesizing protein. Possible S-RNA is a precursor to microsomal RNA. Lipoprotein membranes are associated with the ribonucleoprotein in cells which secrete protein such as the pancreas and thyroid, the lipoprotein being implicated in the secretory process. Evidence has been obtained also that protein synthesis occurs continuously within nuclear chromatin material which is composed chiefly of DNA and protein (25).

The synthesized protein particle could be visualized as rolling off an RNA template or emerging from the microsome as chains of amino acids held together by peptide linkages. This linkage is the result of loss of water, thereby forming a bond between an amino group of one amino acid and the carbonyl group of another. Thus the reverse of synthesis is the addition of water, a process called hydolysis which liberates free amino acids from these polypeptide chains. Each type of protein is characterized by the particular sequence of amino acids in that chain (18). It is interesting to speculate on the mechanism that could account for the synthesis of so many different types of proteins required by the living system. Since the sequence of amino acids is thought to be associated with a template in the microsomes, a large variety of fixed templates in microsomal interfaces would be required. However, a more dynamic mechanism could be visualized where the sequence of arrangement of amino acids along the chain is the result of a meshing, so to speak, of peptide-forming reactions which can be altered by shifts in the physicochemical environment. The synthe-

hydrate metabolism some of the amino acids re-enter the larger pool of the body, thereby becoming available for oxidative deamination in the liver. The significance of this oxidative deamination will be discussed later. Other hormones may influence amino acid transport. Noall, Riggs, Walker and Christensen have employed an unmetabolizable amino acid, aminoisobutyric acid (AIB) to study transportation mechanisms, this amino acid apparently undergoing transfer in a normal fashion (17). For example, estradiol intensified the concentration of AIB by the uterus but not by the liver while hydrocortisone increased transport in the liver. Increased tissue concentration of amino acids also accompanied the introduction of epinephrine or the growth hormone. The important point to be emphasized here is the dynamic state of transportation of amino acids from one cellular pool to another and the effects of this dynamic state on the overall utilization of dietary amino acids for growth and maintenance of tissue proteins.

PROTEIN SYNTHESIS

Only a brief general discussion of the mechanism of protein synthesis can be recorded here, inasmuch as changes in viewpoint and detail must accompany the rapid increase in knowledge that is now taking place. Early viewpoints on protein synthesis and protein turnover have been nicely reviewed by Haurowitz (18). The immediate precursors of new protein are believed to be the free intracellular amino acids. The synthesis of protein is a fast reaction, the amino acids being assembled into a protein molecule or particle in a few minutes, possibly in some cases in less than a minute.

Beginning with the work of Caspersson (19) and Brachet (20), considerable evidence has been presented indicating that nucleoproteins are involved in this rapid protein synthesis. Nucleoproteins are combinations of proteins with ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). The DNA complex is found in the nucleus of cells and its function seems to be that of ensuring the individuality of the chromosomes. The ribonucleic acid (RNA) exists in a free form in the cytoplasm and is also a part of cytoplasmic protein structures called microsomes.

The central role of soluble RNA (S-RNA) in protein synthesis

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The central role of soluble RNA (S-RNA) in protein synthesis

TABLE I

Indispensable (and Related) Amino Acids Expressed as Milligrams Per Gram of Nitrogen in Various Protein Sources (26, 36)

Protein Source	*Isoleucine*	*Leucine*	*Lysine*	*Phenylalanine*	*Tyrosine*	*Sulfur containing Acids*		*Threonine*	*Tryptophan*	*Valine*
						Total	*Methionine*			
Egg white	403	548	450	371	208	446	200	305	72	466
Casein	397	626	506	358	322	228	203	241	60	460
Beef	323	488	537	243	185	253	169	278	63	521
Fish	317	474	549	231	159	262	178	293	62	527
Soy bean	333	484	395	309	201	197	86	247	86	524
Peanut flour	259	442	218	307	181	142	52	174	49	293
White flour	262	442	126	322	174	192	78	174	69	262
Wheat gluten	284	450	126	315	174	252	104	173	49	266
Corn meal	293	827	179	284	385	197	117	249	39	527

sis of the protein thus depends upon an adequate supply of each kind of amino acid in proper proportions. If a single kind of amino acid was missing the protein could not be synthesized. For that reason the proportions and quantities of the indispensable amino acids are primary factors in controlling synthesis.

CATABOLISM VS. ANABOLISM

The dynamic state of the living system is the result of a precise interplay between breakdown (catabolic) and synthetic (anabolic) processes (26). Some of the amino acids come into contact with catabolic processes resulting in the oxidative deamination and in the formation of the nitrogenous waste product called urea. The magnitude of the excretion of urea may be taken as a measure of the amino acids not utilized from the metabolic pool for synthetic purposes. Since some tissue proteins are continually hydrolyzed to contribute amino acids to the metabolic pool, urea is formed whether or not amino acids are provided by the diet. The urea that arises from oxidation of amino acids of body protein origin is sometimes called endogenous, while urea coming from the oxidation of amino acids from dietary sources is said to be exogenous. This concept of endogenous and exogenous protein metabolism was proposed by Folin (27), and has proven quite useful in studies in nutrition. Folin also emphasized the excretion of another nitrogenous waste product called creatinine, which is essentially independent of dietary protein intake. Creatinine is the end product of energy metabolism in the muscle cells and the excretion of this compound is a valuable indication of active muscle mass.

It was pointed out previously that a deficiency in any one of the amino acids below requirements for protein synthesis would result in limited anabolism. Thus the proportion of one amino acid with respect to the others, sometimes called balance, is important for optimum synthesis of cellular proteins. Indeed, an adverse effect upon the living system can result from an imbalance of amino acids, a subject that has been studied extensively by Elvehjem, Harper, Sauberlich, Salmon and associates (28, 29, 30). The expression, pattern of amino acids emphasizes the importance of balance. The patterns of indispensable amino acids which are found in various

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Beef	323	488	537	243	185	253	169	278	63	321
Fish	317	474	549	231	159	262	178	283	62	327
Soy bean	333	484	395	309	201	197	86	247	86	328
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dietary protein sources are recorded in Table I. These patterns represent the amino acids presented to the body if the dietary protein source is digested adequately. Data will be presented later in the monograph to indicate that the pattern of indispensable amino acids presented to the body by egg protein is excellent for growth and maintenance. Thus the egg protein pattern could be selected as a reference to determine deficiencies of indispensable amino acids in other dietary proteins (31-35). Accepting the egg pattern for a reference, all of the other proteins in Table I would be deficient in sulfur containing amino acids. Peanut flour, white flour, wheat gluten, and corn meal would be deficient in lysine. These same protein sources would also be deficient in tryptophan. An attempt will be made, however, to develop a reference pattern from data on growth and maintenance in man and animals to compare it with the egg pattern and with the provisional reference pattern presented recently by the Food and Agricultural Organization (36).

LABILE PROTEIN RESERVES

If an animal is fed a protein-free diet there is a decrease in the amount of some of the tissue proteins which are considered labile and function at least in part as "reserves." This concept of reserves was emphasized by Whipple and his associates (10) and it has proven very useful for the interpretation of shifts in body proteins which accompany changes in diet or physiological states. True, there are no special cells to store proteins as fat is stored in the body, but there are a number of proteins that increase or decrease with a corresponding increase or decrease in dietary protein intake. Plasma albumin, for example, is such a protein. There is a maximum amount of plasma albumin, a maximum which cannot be increased by feeding excess dietary protein whereas a deficiency in intake, whether in quantity or quality, is usually reflected by a decrease in albumin. Some plasma globulins, on the other hand, do not decrease in the protein-depleted individual and are therefore not classified as labile reserves. This does not mean, however, that metabolism of these globulins is static. On the contrary, they may be broken down and resynthesized at a relatively rapid rate,

but the net result of the balance between catabolism and anabolism is either no change or even an increase in certain globulin fractions during depletion (37). Possibly a labile reserve like plasma albumin contributes to the pool of amino acids to conserve other proteins like globulins. Data to support this concept have been obtained. For example, an animal was given methionine tagged with radioactive sulfur (S^{35}); this tag appeared in a few hours in most of the plasma proteins. The tag, however, accumulated in higher concentrations in certain globulin fractions in the depleted than in the normal animal (38-40). The lives of tissue proteins vary, (41-44) some being very short, some longer and still others, like one-way streets, being formed from but contributing little or nothing to the metabolic pool.

The black bars in Figure 1 illustrate the total protein in various tissues in adult rats depleted by feeding a protein-free diet. These data were taken from Wainio *et al* (50). The muscle was represented by the gastrocnemius and the heart by the ventricle, including the atrioventricular septa and the associated valves. The kidney was free of the capsule. The brain was scooped out with a rounded end of a scalpel to the foramen magnum. Since the rat restricted food intake when given a protein-free diet, a group of these animals were pair-fed this diet with one containing 18 per cent casein. The bars with slanted lines illustrate the increase in tissue protein associated with feeding casein in the restricted diet. The white bar illustrates the tissue protein in animals fed the casein diet ad lib. The total protein of the heart, liver, muscle and kidney were increased in animals fed casein. Depletion or repletion, on the other hand, did not affect the total protein in the brain.

The labile reserves, therefore, are found in many cells of the body although some cytoplasmic proteins, for example, of the liver and the gut are particularly susceptible to protein depletion (45, 46). Since tissue proteins are, in general, components of enzyme systems, some of these catalysts are reduced in activity, some remain unchanged, and others increase in activity during depletion (47-51). Protein depletion, for example, had no effect upon unit enzymic activity of the brain but did lower the activity of many of the enzymes of kidney, skeletal muscle and spleen from 10 to

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changes which are most easily demonstrated in the blood and liver (52-55). An excellent and thorough review of protein nutrition and enzyme changes in man has been written by Waterlow (55). He points out that the pattern of enzymatic activity in different organs must be changing as depletion proceeds. Thus consideration of these patterns, together with alterations in biochemical responses should lead eventually to a better understanding of the significance of the labile protein reserves.

These and other data suggest that the so-called reserves are utilized during periods of amino acid deficiencies to help maintain certain essential protein structures in a dynamic state. They may also be needed to correct for imbalances when the dietary intake of protein is either too high or too low. The problem of estimating optimum reserves is one of the most important in nutrition.

PROTEIN MALNUTRITION

Many illnesses and forms of malnutrition result in an alteration in amino acid and protein nutrition and metabolism. Data have been presented illustrating changes in the blood and tissue proteins that are common to animals with certain types of tumors (56-60), with pneumonia (26, 59), with lack of adequate dietary protein (26) and with any change in physiological state that results in an alteration of enzyme systems or structural elements associated with the protein reserves of the body (26). To give one illustration, a lack of sufficient riboflavin in the diet can reduce the activity of certain oxidative enzyme systems, of which this vitamin is an essential part. But the enzyme is also a protein so that a deficiency in dietary amino acids can have a similar biochemical effect as the deficiency in riboflavin. Either the vitamin or the protein deficiency can reduce the ability of the liver to oxidize or detoxify certain chemical compounds (60).

A deficiency in dietary protein is prevalent throughout many areas of the world, a deficiency that results in various forms of protein malnutrition (61, 62). Such a deficiency is most detrimental to children during the period of rapid growth when the reserves may be considered as relatively low with respect to the needs for the stress of growth. At one extreme this malnutrition is the result of a re-

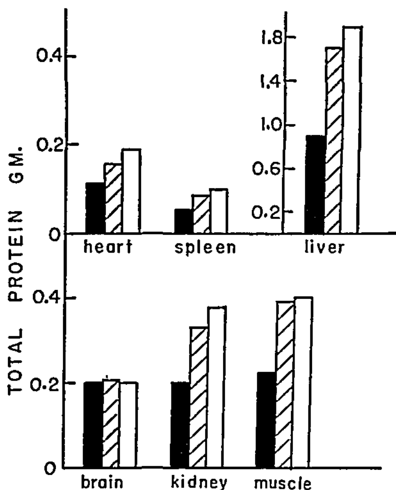


Figure 1. Total protein in tissues of rats fed a protein-free diet (black bars); pair-fed 18% casein diet (bars with slanted lines); fed ad lib casein diet (white bars) (50).

20%. In general, the data reveal that total protein and enzymes of the liver are the most labile while the total protein and the enzymes of the brain are most resistant. The total protein and enzymes of the ventricle of the heart are more resistant than those of the kidney, spleen, or skeletal muscle and almost as resistant as those of the brain (50).

Alterations in the activities of enzyme systems have been reported also in children suffering from protein malnutrition,

changes which are most easily demonstrated in the blood and liver (52-55). An excellent and thorough review of protein nutrition and enzyme changes in man has been written by Waterlow (55). He points out that the pattern of enzymatic activity in different organs must be changing as depletion proceeds. Thus consideration of these patterns, together with alterations in biochemical responses should lead eventually to a better understanding of the significance of the labile protein reserves.

These and other data suggest that the so-called reserves are utilized during periods of amino acid deficiencies to help maintain certain essential protein structures in a dynamic state. They may also be needed to correct for imbalances when the dietary intake of protein is either too high or too low. The problem of estimating optimum reserves is one of the most important in nutrition.

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duced food intake, an overall starvation which produces a condition called marasmus. At the other extreme, the protein malnutrition is the result of an inadequate protein intake in the presence of relatively high calorie intake, a condition sometimes referred to as "sugar baby," and may be considered as an example of classical kwashiorkor. There are, of course, all gradations between these two extremes with those in between sometimes being classified as marasmic kwashiorkor. The syndrome of kwashiorkor generally consists of retardation in growth, muscular wasting, edema, accumulation of body fat with a marked fatty liver, depletion in protein reserves, dermatitis, changes in the hair and apathy with other psychic changes. The syndrome for marasmus is similar except that there is marked weight loss with greater retardation of growth, accompanied by muscular atrophy but with less or no clinical edema or fatty liver. An excellent description of these forms of malnutrition is presented in a paper by Béhar *et al* (63).

The syndrome of kwashiorkor is one of the best illustrations of the effects of protein malnutrition. Brock, Hansen, and Howe (64, 65) have demonstrated that a cure of this disease can be initiated with a mixture of amino acids given in the presence of adequate calories. Vitamins and other essential nutrients were needed, of course, to carry the cure to completion. The group working with Scrimshaw at the Institute of Nutrition of Central America and Panama (INCAP) (63) have also revealed the protein nature of this deficiency by their studies on the initiation of cure as well as its completion by feeding deficient vegetable proteins supplemented with amino acids. The world wide nature of this problem can be illustrated by the numerous papers on protein malnutrition and, particularly kwashiorkor, which have appeared in recent years. Among these are the authoritative survey of the disease by Brock and Autret (66), the studies of Platt (67, 68), of Brock, Hansen, Walker and others in South Africa (64, 65, 69, 70) of Sénécal and colleagues in West Africa (71), of DeMaeyer *et al* in Central Africa (72, 73), of Dean, Jelliffe and colleagues in East Africa (74-77), of Ooman in Indonesia (78), of Gomez, Cravioto *et al* in Mexico (79, 80), of Patwardhan, Sreenavasan, Gopalan and others in India (81-83), of Waterlow, Garrow *et al* in Jamaica (39,

40, 55, 84, 85), of Sebrell and Severinghaus *et al* in Haiti (86), of Autret and Béhar (87), and Waterlow and Vergara in South America (88), of Scrimshaw, Béhar, Ascoli, Arroyave, Bressani, Mendes, Tejada, Viteri, and others of INCAP (63, 89-94) and of a number of conferences held throughout the world (36, 95, 96). Many of the conditions associated with the protein malnutrition of kwashiorkor will be discussed in the remainder of the monograph.

RESISTANCE TO DISEASE

One of the most interesting and important problems that has been presented to the scientist is the relationship between protein malnutrition with its reduction in protein reserves and resistance to disease. Scrimshaw has presented an excellent review of this subject in which he emphasizes that the presence of kwashiorkor reflects the prevalence of protein malnutrition, often unrecognized, in the population particularly of the post-weaning and pre-school years. The stress of infection is also a depleting mechanism so that infection is often the precipitating factor leading to the recognition of protein malnutrition. Furthermore, as he points out, many of these children die as a result of an acute infection which would not normally be fatal to a well-nourished child (97).

Dubos (98-100) has demonstrated an increased susceptibility of mice to certain experimental infections when the quantity or quality of dietary protein is deficient. Susceptibility to infection can change independently of the immunological condition of the host. Thus susceptibility may be correlated in part with the biochemical state of the area receiving the infectious agent. A change in susceptibility to transplanted tumors with a shift in dietary protein in rats is discussed later under abnormal growth. The induction time of the tumor was altered by changing the amount and kind of dietary protein intake. The induction time, however, was longest and the overall resistance to the growth of some tumors was greatest when the protein reserves were relatively adequate. Under these conditions some of the tumors would even regress.

Cannon (101) has demonstrated that production of antibodies was closely associated with nutrition, particularly with the magni-

tude of the protein reserves and the dietary protein intake. Gemeroy and Koffler (102) similarly found that an adequate protein diet was essential for the production of hyperimmune sera in rabbits. The specific nature of some forms of resistance, however, has been emphasized by Schneider (103) who cautioned against accepting a concept of pan resistance where a single type of diet or physiological state will result in resistance to all types of infections. Pertinent data have also been published by Sriramachari and Gopalan. (104).

Specific effect of diet upon susceptibility and resistance of the host has been demonstrated by the studies of Stauber and his associates (105, 106). They have shown that *Leishmania donovani* developed most rapidly in cells in the liver of the hamster fed a normal diet. Feeding a protein-free diet reduced the rate of growth, a reduction that could be associated with changes in the intra-cellular media such as the depletion of certain liver cells necessary for growth of the organism. The parasite grew less in cells in the liver of the mouse than in the hamster. Feeding a protein-free diet to the mouse or a pyridoxin deficient diet increased rather than decreased the rate of growth of *L. donovani*. Such a response might be the result of a decrease in the natural resistance of the mouse to this parasite. A pantothenate deficiency reduced the rate of growth of the parasite for a time but eventually, as the deficiency increased, the parasite took over and grew rapidly.

Protein depletion undoubtedly also produces shifts in the endocrine balance which would affect susceptibility and resistance to disease. Leathem and others have demonstrated marked effects of protein nutrition upon endocrine function and the physiological response to hormones (107, 108). Data have been presented, for example, to indicate that the growth hormone promotes nitrogen retention, particularly when the supply of dietary protein or carbohydrate is restricted (109).

The possibility that there is an optimal balance between protein stores and other constituents of protoplasm which decreases susceptibility to most infections and provides a maximum ability to recover from them should be emphasized.

SPECIFIC AMINO ACID DEFICIENCIES

Early in the development of the science of nutrition, the importance of protein, carbohydrate, fat, minerals and water in the diet was recognized. For many years, however, emphasis was placed upon the energy requirements of the living system and the caloric value of foods. This emphasis was natural because studies of the living system all revealed the fundamental need for energy. Deficiency diseases associated with lack of food factors, called vitamins, were discovered later and the identification of specific deficiencies became of major interest to the nutritionist. The results of this interest have been revolutionary in correcting dietary errors throughout the world. In the meantime, the importance of the indispensable amino acid in the diet was revealed and there was a search for deficiency diseases that, like the vitamin deficiency, could be recognized as the result of an insufficient intake of some particular amino acid. It is not surprising that such specific deficiency diseases have not been clearly revealed because of the interrelationship of one amino acid to the others in the synthesis of tissue proteins and enzymes, an interrelationship that includes many of the other essential nutrients such as the vitamins and minerals (110, 111). There are, however, specific roles for some particular amino acids. Tryptophan, for example, has at least three important functions: (a) Part of the niacin requirement is derived from this amino acid (112, 113). (b) It is a precursor of serotonin (114). (c) It is an indispensable amino acid for the synthesis of cellular proteins of various kinds. The deficiency disease black tongue in dogs and pellagra in man were cured or prevented by the proper treatment with niacin but because of the interrelationship between tryptophan and niacin, pellagra may be considered as a lack of both the amino acid and the vitamin (115-119).

Methionine is another example of an indispensable amino acid with several functions. Besides being an important constituent of tissue proteins, it is the precursor of cystine and is also a methyl donor (120, 121). The methyl group, for example, of methionine is utilized through transmethylation to convert nicotinamide into N'-methyl nicotinamide (122, 123), to complete the synthesis of adrenalin (124) and to convert guanidinoacetic into creatine (125,

126). A combination of methionine and guanidinoacetic acid has been used to increase the rate of repletion of plasma albumin in protein-depleted dogs fed a diet containing casein (37, 127).

Phenylalanine, also an indispensable amino acid, is another example of the varied specific roles or effects of an amino acid. The clinical syndrome of phenylketonuria develops in children as a result of the absence of the enzyme catalyzing the conversion of phenylalanine into tyrosine (128-130). Excess phenylalanine in the metabolic pool results in serious disturbances, some of them involving the central nervous system. A biochemical improvement in phenylketonuria results from feeding a diet low in phenylalanine but with sufficient to meet the minimum needs for maintenance of life (131-135). Excellent reviews of these special aspects of amino acid metabolism are included in the papers by Huisman (134) and by Tower (135). The possibility of using amino acids to effect rates of intermediary reactions for therapeutic purposes are very attractive and should be studied extensively.

With the above introduction as a background, a more detailed analysis of some phases of protein nutrition and metabolism will be developed around the following topics: (a) Digestion, (b) Nitrogen Balance, (c) Proteins and growth, (d) Proteins and maintenance, (e) Proteins and calories, (f) Nutritive value and problems of supplementation, (g) Plasma and liver proteins. Illustrative data will be restricted mostly to results obtained in the Bureau of Biological Research.

DIGESTION

THE GASTRO-INTESTINAL tract of man or of animals is a complicated system possessing many of the characteristics of the dynamic state described for the animal body. The body secretes or empties into the tract various fluids, rich with digestive enzyme systems, together with waste products and cellular debris. A bacterial flora is a normal feature of the intestinal tract and the contents of the small intestine provide an excellent medium for the growth of microorganisms. Consideration must be given to the flora if the role of the gastro-intestinal tract is to be well understood. For example, Anderson (136) studied a group of dogs made parasite free and fed a semi-synthetic diet containing casein, sugar, dextrin, starch, lard, minerals, water, vitamins, and agar (60). Yeasts, colifrom bacteria, entercocci, lactobacilli, proteolytic bacteria, were found distributed throughout the intestine. The upper portions of the tract contained millions of bacteria per gram of contents, the number being associated with the abundance of food material. The irregular moist intestinal wall harboured large numbers of very active bacterial cells which served as an inoculum for new supply of food and the numbers of bacteria increased very rapidly as the intestinal contents were directed toward the colon. In the colon, the multiplication of microorganisms continued to be rapid. The greater numbers in this region and in the feces were also the result of concentration by removal of water. The numbers of bacteria present in the lower portion of the small intestine, colon and feces were greater in dogs fed the diet with protein than in dogs fed a protein-free diet. Certain of the bacteria could be a source for vitamins and other nutrients to the host especially in animals that ingest their feces. The importance of prevention of coprophagy in the rat in studies on nutritional requirements has been emphasized by Barnes and associates (137).

Dietary proteins are hydrolyzed to amino acids in this dynamic state of the intestinal tract, some more rapidly than others. Thus an

amino acid pool is generated in the gut, a pool that is in contact with the bacterial flora as well as with the host. Such a pool would be altered by the absorptive and synthetic processes of the host and the bacteria. Recently Nasset (138) has presented evidence for the alteration of the dietary pattern of amino acids by the dynamic state of the intestinal tract. The data in the literature, however, demonstrate that the pattern of free amino acids in the plasma can reflect post absorptively any deficiency of the dietary protein pattern (139, 140) and that the orderly hydrolysis of proteins in the intestinal tract is an excellent way to present the amino acids to the system. The same pattern of amino acids, for example, is utilized better for protein synthesis if it is fed in the form of an easily digested protein rather than a mixture of amino acids fed intravenously (141, 142). The rate and degree of digestion of the dietary protein, therefore, is of first importance in any analysis of the nutritive value of the diet.

Gupta *et al.* (143) found that the nitrogen disappeared from the gastrointestinal tract of rats, at about the same rate whether they were fed a diet containing casein, beef or a mixture of amino acids. Zein, however, was emptied more slowly from the stomach than were the other amino acid sources and even tended to accumulate in the intestine. Geiger *et al.* (144) also pointed out that the disappearance and the distribution of nitrogen between stomach and intestine is a function of the nature of the protein. The emptying time from the stomach and rate of digestion in the intestine may indeed alter the pattern of amino acids liberated and absorbed from that calculated from chemical analyses of the dietary protein. The work of Geiger *et al.*, (145, 146), Cannon *et al.* (147), and others have demonstrated that the time of absorption of each amino acid with respect to the others is important in establishing the pattern for anabolic activity in the body.

The nutritive value of a protein could be impaired if most of the amino acids were liberated and absorbed rapidly during early stages of digestion while an indispensable amino acid was liberated and absorbed more slowly (148-150). Howe *et al.* (151) fed a low intake of cottonseed meal, supplemented by indispensable amino acids to make up for the deficiencies in the protein. This

supplemented protein had a low nutritive value, indicating that the pattern of amino acids absorbed was not the one predicted from the diet. Possibly the free amino acids were absorbed more rapidly than those presented to the body by the slowly digesting cottonseed meal. A similar experiment with casein resulted in the nutritive value which was predicted from the amino acid pattern available in the diet. In general, experiments involving supplementation of dietary protein result in nutritive values that can be predicted from the pattern of amino acids in the diet, results which suggest that the rate of digestion of many dietary proteins to amino acids is rapid and efficient. The studies of Rosenberg (153) and others, for example, on supplementation of deficient proteins with amino acids have demonstrated that the deficiency of most dietary proteins can be overcome simply by adding the proper indispensable amino acid in adequate but not excessive quantities.

The absorption of amino acids from the intestinal tract into the blood stream is example of an active transport. Paine *et al.* (154) studied intestinal absorption of methionine and histidine by the chicken with a fistula. The L isomers were absorbed more rapidly than were the D isomers. The L isomers appeared to be absorbed from the intestine by a common specific transport mechanism. These and other studies indicate that the rate of passage of amino acids through the intestinal wall is mostly governed by the rate of uptake by the epithelial cells from the lumen. Orton and associates (155), using a blind loop of the human intestine, found that absorption of amino acids from the gastrointestinal tract is competitive. Thus the rate of absorption of individual amino acids will be determined in part by the proportion of the other amino acids that are presented simultaneously to the absorptive mechanism. Digestion of proteins and absorption of amino acids from the intestine, therefore, is a process of primary importance in the presentation of a pattern of amino acids in the body.

Intestinal parasitism and diarrhea can reduce the amount of digestion and absorption (97). Depletion in body proteins results in a marked reduction in the protein of the intestinal tract (45) and in the activity of digestive enzymes (156). These replete rapidly, however, and in the child with kwashiorkor, repletion

results in a rapid return to adequate digestion of dietary protein and absorption of amino acids (63).

Some forms of processing of foods may improve the rate of liberation of amino acids in the intestinal tract but there are others which can reduce digestibility and may destroy certain essential amino acids (148, 149, 150). Processing may also improve foods by denaturing toxic proteins or antienzymes present in raw foodstuffs (159,160). Indeed, one of the functions of food technology is to prepare foods so that they will be easily digested and will present an adequate pattern of amino acids to the body (161).

Since nitrogen is the characteristic element in amino acids, the nutritionist expresses the absorption of a mixture of dietary amino acids in terms of nitrogen. Thus digestibility D (actually the fraction of nitrogen absorbed) is defined as:

$$D = \frac{A}{I} \qquad \text{(I)}$$

where A is the amount of dietary nitrogen absorbed into the body and I is the total dietary nitrogen intake. The nitrogen lost in the feces is assumed to be composed of unabsorbed food nitrogen plus metabolic nitrogen or fecal nitrogen of endogenous origin. Much of the food nitrogen, however, may be transformed into bacterial protein nitrogen. The assumption is made that food nitrogen in the feces can be calculated from the following equation:

$$\text{Fecal food nitrogen} = (F\text{-}F_0) \qquad \text{(II)}$$

where F is total fecal nitrogen and F_0 is fecal nitrogen of endogenous origin. The value of F_0 is often calculated by feeding a protein-free diet. Absorbed nitrogen A can then be calculated as follows:

$$A = I - (F\text{-}F_0) \qquad \text{(III)}$$

This method of calculation assumes that F_0 is constant, independent of nitrogen intake, an assumption that is not always valid for every type of animal or individual under all physiological states, (162). Nevertheless, this calculation is a good approximation for absorbed nitrogen in man and many other animals under most conditions (163).

NITROGEN BALANCE

SIMILAR TO DIGESTION, over-all protein nutrition is often described in terms of nitrogen (164, 165). Such a description is possible because of the presence of nitrogen in amino acids and their statistical distribution in the various molecules or particles of protein. The body proteins, for example, of the animal contain approximately 16% nitrogen. Thus multiplying the nitrogen content of tissues by the factor 6.25 gives a good estimate of the amount of protein in those tissues.

The dynamic state of protein metabolism in animals, discussed previously, makes possible the attainment of a balance between nitrogen intake and the nitrogen excreted as follows:

$$B = I - (U+F+S) \qquad (IV)$$

where B is nitrogen balance, I is nitrogen intake, U is urinary nitrogen excreted, F is the excretion of fecal nitrogen and S is the loss of nitrogen through the skin in man. The value for S in man has been reported as ranging from 0.1 to 0.4 grams per M^2 per day. Since the loss of nitrogen from the skin is difficult to measure, it is often neglected in nitrogen balance studies in man. The loss of S through the skin is not a significant factor in establishing balance in animals such as the rat or the dog. The largest errors, however, that can enter into nitrogen balance measurements, as pointed out by Wallace (166) are in the estimations of nitrogen intake and excretion. There is always a tendency to overestimate intake (I) and to underestimate the nitrogen excreted (U + F) so that the calculated balance may be excessive.

If nitrogen intake (I) is just equal to excretion (U+F+S), then the individual is said to be in nitrogen equilibrium. The assumption is often made that an individual in nitrogen equilibrium is maintaining the status quo. Subsequent data will emphasize that nitrogen balance is an algebraic sum of gains and losses throughout all the tissue compartments of the body so that some tissues may be gaining while others are losing nitrogen in animals

with an over-all zero nitrogen balance. In general, however, the body adjusts to a given nitrogen intake, thereby seeking a stable state which results in the establishment of an overall nitrogen equilibrium.

If intake is greater than the total excretion of nitrogen then the body is gaining in protein. Children and growing animals should be in positive balance. Adults also should be in positive balance instead of equilibrium, if, for any reason, the tissue proteins have been depleted and need to be repleted. It should be emphasized that positive balance does not mean that all tissue protein compartments are gaining nitrogen at the same rate, indeed some tissues could be in negative balance even though the balance is positive for the individual as a whole. Such an imbalance in the growth of the various tissues was observed in an exaggerated form by the effect of certain neoplasms on the body of animals. For example, these tumors will grow and be in positive balance while some normal tissues are losing protein (58). Normally, however, the metabolism of the body seems to be directed toward a balance so that, given an adequate diet, growth of new tissues or repletion of depleted tissues is a balanced process.

If intake is less than the nitrogen excreted, the body is losing nitrogen and is said to be in negative nitrogen balance. It was pointed out previously that the body is continually losing nitrogen through metabolism of tissue proteins and associated nitrogenous constituents, nitrogen that must be replaced daily by the diet to maintain nitrogen equilibrium. Terroine (167) and Sorg-Matter (168) and others proposed that the minimum endogenous nitrogen excretion in various animals was equal to approximately 2 mg of nitrogen per basal calorie. A similar value for maintenance in man was used by Bricker, Mitchell, and Kinsman (169). Hegsted (170, 171) also used a value of this order to calculate the basal nitrogen requirements for children and adults. The cross hatched area in Figure 2 (172) illustrates this daily loss of body nitrogen in terms of negative balance, nitrogen that must be replaced by dietary nitrogen to maintain nitrogen equilibrium. These data are based on average requirements for maintenance in a population of boys and young men and represent approximate calculations from the

relationship described between basal calories and endogenous nitrogen loss. The data are essentially the same as those presented by the Committee on Amino Acids of the Food and Nutrition Board of the National Research Council (173) and by Hegsted (170, 171), but presented in a different way. These data are presented as approximations to emphasize the rather regular increase in both body nitrogen catabolism and dietary requirements that are associated with growth until the adult state is reached. Such a regular increase in the requirements may represent one of the most fundamental features of growth. The requirement for maintenance will, of course, reach some maximum value in adulthood so that the lower curve in Figure 2 would become parallel to the X axis. This regular increase in requirement, however, might continue for a time, even though growth has almost ceased at twenty-one years, so that the maximum would vary with the magnitude of the protein reserves developed within the body fabric. Recent studies demonstrated that older men were in negative nitrogen balance when fed a diet that produced nitrogen equilibrium in younger men (174).

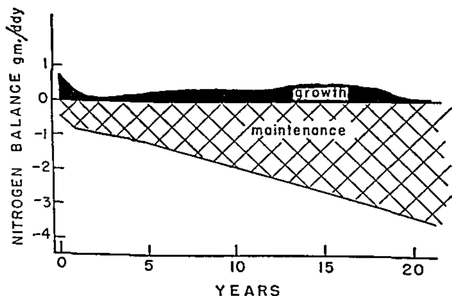


Figure 2. Minimum nitrogen for maintenance and growth. Published with permission of 5TH Int. Cong. on Nutrition (172).

The daily requirement for maintenance can be converted into protein by multyplying by the factor 6.25. For example, if 4.5 grams of nitrogen are lost daily in the adult this loss would be equivalent to 28 grams of body protein which should be replaced by dietary amino acid scources. However, the amount of endogenous metabolism varies with the physiological state of the individual and the magnitude of the labile protein reserves. If those reserves are full and catabolic activity is high, the loss of body nitrogen would be greater than the so-called basal values illustrated in Figure 2. For that reason it is difficult to select a single fixed value for maintenance. If, for example, the body looses 28 grams of protein, the diet would need to provide this amount of well digested protein with an ideal pattern of amino acids for synthesis plus some for wastage. If the labile protein reserves are higher and there is greater endogenous catabolism with loss of body nitrogen, 40 grams of a dietary protein with an ideal pattern of amino acids may be needed to meet the requirements of a population of individuals. Furthermore, if the pattern of dietary amino acids is not ideal for anabolism, more dietary protein would be required to correct for the deficiency in the pattern. Thus the 70 grams of protein per day or the 1 gram of dietary protein per day per kg. of body weight, recommended for maintenance in the adult American male, is a realistic value for an individual eating an average mixed diet. If it is assumed that endogenous catabolism represented by maintenance is, in part, a homeostatic mechanism *to help meet internal and external environmental stresses, then* the magnitude of this area under zero nitrogen balance should be developed to an optimum but that optimum is unknown.

The darkened portion in Figure 2 is an attempt to illustrate approximately, the average amount of nitrogen, expressed as positive balance, that may be retained for growth. These data were calculated by assuming certain body weight gains with from 16 to 18% protein in these gains. According to these calculations the infant from birth to three months would need approximately 0.73 grams of dietary nitrogen for growth, this amount of nitrogen, multiplied by 6.25 being equivalent to 4.6 grams of protein per day, or about 1 gram of protein per day per kg. of body weight. The

data in Figure 2 also suggests that the infant at this age would need 0.45 grams of nitrogen per day for maintenance which would approximately equal 0.63 gram of protein per day per kg. of body weight, a minimum value. Thus the infant would require 1 gram of protein for growth and 0.63 gram for maintenance or a total of 1.63 gram of protein per day per kg. of body weight. If 10% is allowed for wastage, the minimum requirement would be approximately 1.8 gram. Obviously, a higher intake would be required for some infants than for others and the intake would need to be increased to take into consideration the efficiency of utilization of the dietary protein fed. Variations among individuals is a fundamental characteristic of all living systems. Since normal individuals can take care of moderate excesses of proteins recommended allowances in the diet are often based upon a quantity that should be adequate for the majority of individuals in a population. The recommended allowance, for example, for a population of infants could be considerably higher than the value mentioned above (175, 176). This quantity, however, can vary throughout the world where various populations may be in different physiological states or adapted to different diets (177, 178).

Various factors which effect protein requirements at different ages have been reviewed recently in several Symposia. Take, for example, the Symposium sponsored by the N.Y. Academy of Science (179). Stearns *et al.* (180) discussed the protein requirements of children from one to ten years of age. These authors emphasized the need for an adequate intake of dietary protein to develop the full quota of cellular proteins, including those which have been defined here as protein reserves. They also considered the differential characteristics of growth indicated by the curves in Figure 2. Thus the rate of growth of skeletal musculature could dominate early protein requirements, growth of muscle and protein reserves could be most rapid during pre-school years. Stuart *et al.* (181) considered the importance of recognizing individual differences. Hence they emphasized the need to take into consideration the dietary history of a child to determine protein requirements. Johnston (182) reviewed the requirements for adolescents and suggested that the optimal intake of protein was approximately

15 per cent of an adequate caloric intake. Watkin (183), while discussing the nutritional problems of the aged, stated that although the requirement for the aged may be different than for the young, socio-economic factors and diseases have more influence than age per se on the requirement for dietary protein in the senior citizen.

At the Borden Centennial Symposium on Nutrition, Darby (184) discussed the requirements of mother and baby. The value of mother's milk as a reference for determining requirements in the baby was emphasized. The importance of human milk as a reference standard was also emphasized elsewhere by György (185). Scrimshaw (186) pointed out that growth and maturation are slowed after weaning and during pre-school years in most children in under developed countries. Further, it is during this period that mortality from protein malnutrition and infection is highest. Cuthbertson (187) discussed the nutritional problems of the adolescent focusing special attention on the physical, biological and emotional changes which effect appetite and nutritional requirements. A review of the untoward influence of prosperity upon the nutritional welfare of a population by Goldsmith (188) was timely for the average American who tends to overeat. Caloric restriction while maintaining a balanced diet presents a problem to the average adult. The requirements of the older person were discussed by Griffith (189) who pointed out that malnutrition of the aged cannot be separated easily from factors such as economic, medical, psychological and social, factors which are involved in gerontology. Hundley (190) surveyed the effects of habits and environment upon nutrition throughout the world. Sebrell (191) led a discussion on the future developments in nutrition. These authors outlined the magnitude of the problems of nutrition particularly those involving protein and the need to coordinate the sciences of agriculture, food technology, nutrition, and medicine to solve them. The following pages will emphasize some of the problems and basic concepts which were considered most important in the symposia.

PROTEINS AND GROWTH

The term growth is used to refer to different ways in which a living system increases in size, for example, in volume or weight. A detailed analysis of growth, however, should contain data revealing the changes in size, structure, and in composition of the whole animal and of its parts. Some components may grow throughout the life span, others may not but there is a more or less characteristic balance in the development of tissues depending upon diet, environment and internal milieu (180, 192).

GAIN IN BODY WEIGHT

Gain in body weight does not always represent an optimum balanced growth of various tissues or of their components. Puppies, for example, were fed the same protein intake in a semi-synthetic diet but one group received egg protein while another was fed wheat gluten. Both groups of puppies gained the same body weight over the feeding period but the composition of their bodies and their behavior differed markedly. The animals fed egg protein were lean and very active. Those fed wheat gluten were obese, inactive and the development of their skeletal system was delayed. The puppies fed the egg protein gained three times as much body protein as those fed the wheat gluten. Thus gain in weight in these puppies did not correlate with an optimum balanced growth pattern of tissues (193). Gain in weight in infants also may not always be a good measure of balanced growth (180).

Young rats, on the other hand, gained much more weight when fed egg protein than when they were fed wheat gluten, the gain in weight being correlated with the gain in body protein. Indeed the work of Hegsted and Worcester (194) of Howard *et al.* (195) and of Bender (196) emphasize a good correlation between gain in body weight in rats and tissue protein synthesis. The internal controls, together with the relatively rapid growth in the rat, result in a balance between caloric intake and tissue protein synthesis so that

the body composition is kept more constant than in some growing species. The rat will reduce food intake when the pattern of dietary amino acids is one that results in reduced protein synthesis. This does not mean, however, that the rate of increase of some types of proteins, such as the collagens may not take precedence over the non-collagens, especially during marked caloric restriction.

PROTEIN EFFICIENCY AND NITROGEN GROWTH INDEXES

Good correlation between gain in body weight and tissue protein synthesis in the rat has resulted in the use of this animal to measure the nutritive value of dietary proteins. Osborne and Mendel and Ferry (197) defined nutritive value of proteins as gain in body weight per gram of nitrogen or of protein intake, a value that was called the protein efficiency. This efficiency increased with nitrogen intake, reaching a maximum value which was selected as the best point for comparison of one protein with another. The importance of considering the variation of protein efficiency with nitrogen intake was emphasized by Barnes *et al.* (198, 199). The *significance of these variations in protein efficiency are illustrated* by the following data.

Groups of weanling male rats were fed various nitrogen intakes over a period of 28 days during which the gain in body weight was essentially linear with respect to time (161, 200). The gains in weight of the animals fed different quantities of egg protein nitrogen are illustrated in Figure 3 by curve A. The effects of varying casein nitrogen intakes upon gain in weight are illustrated by curve B. The curve C records body weight gain in rats fed various intakes of a soy bean protein. Curve D illustrates growth in animals over the period of twenty-eight days while they received various intakes of cottonseed meal protein while curve E records data obtained while feeding different amounts of wheat gluten or of semolina.

The curves in Figure 3 could be considered as essentially exponential, expressing the "law of diminishing return." Thus all of the curves approach an intake which provides maximum gain in weight, this intake being least for egg protein and never being reached in animals fed wheat gluten. Indeed the ability of a protein

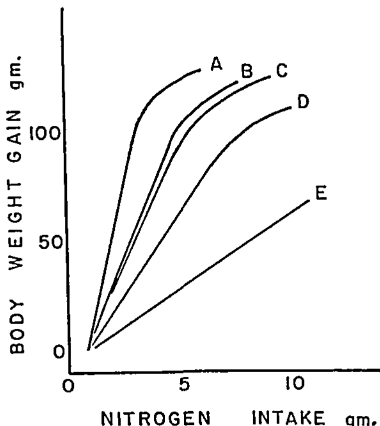


Figure 3. Body weight gain in rats fed different amounts of protein sources from weaning over a period of twenty-eight days. Egg protein, A; casein, B; soy protein, C; cotton seed meal, D; wheat gluten or semolina E (161).

to produce this maximum growth is one measure of its dietary usefulness. A protein, for example, could produce rather satisfactory increase in body weight at lower intakes but never produce a maximum growth at higher intakes because of toxic action or some other form of inhibition. The lower portions of these curves in Figure 3 are essentially linear and the slopes have been called nitrogen-growth indexes. These indexes were egg protein, 30; casein, 22; soy bean, 20; cottonseed meal, 14; wheat gluten, 7; semolina, 7. These values represent a constant gain in weight per gram of nitrogen intake over the linear portion of the curves and could be

called the protein efficiencies of body weight gain. They are not, however, calculated according to the original definition of protein efficiency which included the total nitrogen intake for each body weight gain. It should be noted that the curves in Figure 3 do not pass through the origin, a certain amount of nitrogen being necessary for maintenance of body weight. Protein efficiencies, calculated in the usual way rise to a maximum as illustrated in Figure 4 for the data described by curves, A, B, D, and E of Figure 3. Thus when a protein of high quality, such as the protein of egg

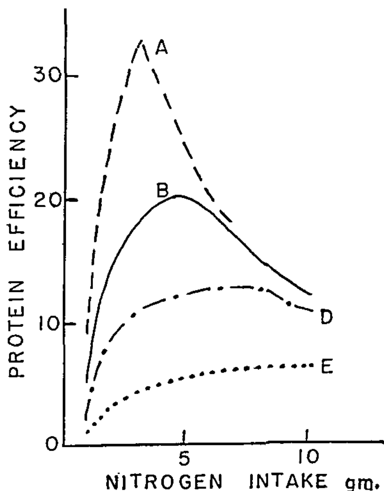


Figure 4. Protein efficiencies (gain in body weight per gram of nitrogen intake) for dietary protein sources illustrated in Figure 3.

is fed, the efficiency rises rapidly with intake to a maximum and then decreases rapidly in value after maximum gain in weight has been reached (curve A). As the nutritive value of the protein decreases, the protein efficiency is reduced and the maximum efficiency becomes more and more a plateau as illustrated by the data for wheat gluten or for semolina (curve E).

A CORRELATION WITH RNA

Reduction in protein anabolism which is associated with reduced nitrogen intake or with a poor pattern of amino acids may be correlated with changes associated with the mechanism for protein synthesis. The assumption has been made that this mechanism involves a protein template containing ribonucleic acid (RNA) (157, 158). Ribonucleic acid may be in dynamic equilibrium with ribonuclease, the enzyme that splits the nucleic acid and which may be a part of a homeostatic mechanism to control protein metabolism. Zigman and Allison (51) demonstrated that serum ribonuclease activity increased from 5.0 $\pm$ 0.25, to 7.3 $\pm$ 0.21, to 18.2 $\pm$ .60 units as the amount of casein in the diet of the rats was reduced from 30 to 12, to 0 per cent respectively. Similarly, the ribonuclease activity of the liver increased from 0.51 $\pm$ 0.03, to 0.61 $\pm$ 0.03, to 0.99 $\pm$ 0.08 units in rats fed these diets with high, medium, and zero casein nitrogen intakes. The ribonucleic acid (RNA) in terms of nucleic acid phosphorus, on the other hand, decreased in the liver from 27.0 $\pm$ 1.4 to 20.2 $\pm$ 0.6 to 13.9 $\pm$ 0.7 μg of phosphorus per gm. of liver as the casein in the diet was reduced from 30, to 12, to 0 per cent. Feeding the highest intake of wheat gluten, represented by 40% of this protein in the diet, produced a serum ribonuclease activity of 11.3 $\pm$ 0.50 units. In the liver this activity was 0.84 $\pm$ 0.03 units and the ribonucleic acid phosphorus was 19.7 $\pm$ 1.1. These values in rats fed the high wheat gluten intake are similar to those fed the lower casein diets (12%) and correspond quite well to the relative weight gains of these animals. This correlation between ribonuclease activity, RNA, and protein synthesis was best in the liver where the labile protein stores are relatively high.

The quantity of deoxyribonucleic acid (DNA), the nucleic acid of the nucleus, is less variable than RNA and can be considered

to represent a fundamental growth in cellular material. Thus the ratios between RNA and DNA in both growing animals and adults are of importance in assessing the effects of diet upon growth and the protein reserves of the body. The RNA/DNA ratios in the liver were 2.3 in animals fed the 30 per cent casein, 1.8 in those fed 12 per cent casein, and 1.4 in those fed the protein-free diet. An experiment by Mendes and Waterlow (201) illustrates the basic value of DNA measurements for the estimation of the degree of depletion in certain protein reserves. They fed weanling rats a low-protein, high-carbohydrate diet designed to simulate the diet of a low economic group of people. These studies revealed that this diet did not support growth, the ratio of nitrogen to DNA being reduced in both liver and muscle. The connective tissue of the muscle, however, continued to increase throughout the period of depletion, a result which emphasizes the selective effect of depletion upon the various tissue proteins. The authors suggest that the ratio of non-collagen nitrogen to DNA in muscle may be a useful measure of the magnitude of depletion. During repletion of these animals, the new protein and DNA formed at about twice the rate that accompanied normal growth. On the other hand, there was evidence of a lag period before the synthesis of new tissues reached its maximum rate in the muscles.

To a certain extent the strength and physical state of the muscle may be associated with the development of collagen and less labile tissue proteins. Data have been obtained to demonstrate increase in tensile strength of muscles in depleted rats (202). Experiments on wound healing in the rat demonstrated a surprising ability to heal and increase the tensile strength of the wound in a protein-depleted rat. Such a healing wound, however, appeared unhealthy and was very susceptible to infection. Filling the protein stores produced a clean dry wound and healthy muscle fibers (203). These experiments illustrate again the possible roles of the so-called protein reserves in providing a reservoir to repair damaged tissues and to increase resistance to a stress such as infections.

GROWTH OF ABNORMAL TISSUES

The effects of various dietary proteins upon induction and growth of transplanted tumors and upon the normal tissues have

been studied in rats (56-60, 204-207). Two of these tumors, a sarcoma and the Walker 256 carcinosarcoma could be considered as nutritionally malignant since they can deplete normal tissues, even growing in animals fed a protein-free diet. These tumors increase serum and liver ribonuclease activity, thereby indicating a depleting type of metabolism (51).

The results of varying casein nitrogen intake, upon the growth of Walker 256 carcinosarcoma and upon the body of the tumor-bearing rat are illustrated by the open circles in Figure 5. The weight of the body was calculated by subtracting the weight of the tumor from the total weight of the tumor-bearing animal. The induction time was shortest and the tumor developed most rapidly in rats fed a total of slightly more than 3 grams of casein nitrogen during the period of tumor growth (14 days). Decreasing or increasing the casein nitrogen intake reduced the rate of development of this neoplasm. The growth of the body, on the other hand, was greatly reduced at the low intakes, but became maximum at a bit over 4 grams of casein nitrogen intake. Body weight gain was somewhat depressed at the highest intakes of casein nitrogen. These data help emphasize the value of an optimum nitrogen intake, an optimum that will vary, however, with the type of dietary protein and with the physiological state of the animal.

The data illustrated in Figure 5 also emphasize the importance of the pattern of amino acids presented to the animal body, particularly under stress. Casein is deficient in the sulfur amino acids required by the rat, a deficiency that can be expressed largely in terms of methionine since this amino acid can be converted into cystine, the other sulfur containing amino acid needed for protein anabolism. Adding methionine, therefore, to the casein so that the total sulfur amino acid content of the protein was raised to 420 mg per gm of nitrogen, resulted in reduced rate of development of the tumor and an increased rate of growth of the body (see circles with vertical bars in Fig. 5). The reduced rate of development of the tumor was largely the result of an increased induction time.

Supplementation of the casein with methionine or methionine plus guanidinoacetic acid had a beneficial effect upon the normal tissues in the presence of the Walker 256 or the sarcoma but was not as beneficial in rats with the Flexner-Jobling carcinoma. This

difference in tumors was illustrated also by feeding various dietary proteins to tumor-bearing rats. For example, the Walker 256 and the sarcoma developed at essentially the same rate in rats fed diets containing wheat gluten, casein or beef but were slightly lower in

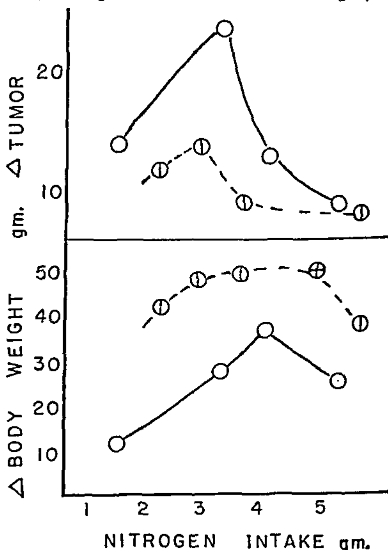


Figure 5. Gain in body weight and in weight of Walker 256 Carcinosarcoma from transplantation to fourteen days in rats fed different intakes of casein (white circles) or casein supplemented with methionine (circles with vertical bars).

animals fed egg albumin. The bodies of the rats, however, grew poorly in animals fed wheat gluten and maximally in those fed egg albumin, the growth being correlated with the nutritive value of the dietary protein. The growth of the Flexner-Jobling carcinoma, on the other hand, was poor in rats fed wheat gluten, and greatest in those fed egg albumin, a response that was similar to normal tissues. Thus tumors may vary in their interrelationship to body metabolic pools, some tumor proteins being more depleted and others not at all by an inadequate intake of amino acids.

In general, protection against the depleting effects of the tumor on normal tissue increased spontaneous regressions and improved the chances of a cure through chemotherapy. Thus nutritional therapy may be an important development together with other forms of therapy in the control and treatment of tumors. The nutritional feature of this therapy, however, can vary greatly depending upon the type of tumor. There is a great need and opportunity for research in this phase of nutritional therapy.

The effects of varying nitrogen intake and pattern of amino acids can also be revealed by other stresses. For example, a hypertension can be produced in rats by removal of one kidney followed by treatment with NaCl and desoxycorticosterone acetate (DCA). One group of rats was fed 18 per cent casein and made hypertensive by this experimental procedure. Another group was treated in a similar manner but fed 40% wheat gluten instead of the casein. Blood pressures increased and there was hypertrophy of the heart, liver, and kidney in both groups. Plasma albumin decreased while serum cholesterol, phospholipid phosphorus, and lipoproteins increased in both groups of animals but the changes were most marked in the animals fed the wheat gluten. Indeed simply removal of one kidney without the hypertension was sufficient stress to produce a significant rise in these lipids in animals fed the wheat gluten (208).

Emphasis in this chapter on growth has been placed upon the development of maximum body proteins and nutritive value was measured in terms of rate of attainment of this maximum. Growth also may be considered a form of stress in that rapid gain in weight

places great demand upon the body and upon the diet. If there is any error in the diet or in the internal milieu, rapid growth may not represent an optimum physiological state. For example, a good pattern of amino acids for tissue anabolism may promote rapid synthesis of body proteins, at the same time some dietary deficiency may deplete the body in an essential component, thereby lowering resistance to stress. It is possible, therefore, for a slower growing animal to show greater resistance to stress than one gaining more rapidly. Sometime ago an attempt was made to develop an ideal semi-synthetic diet for hamsters. This diet promoted maximal growth in hamsters, resulting in large healthy appearing animals. They were, however, very susceptible to a disease which was called "wet spot," a diarrheal disease leading to dehydration, inanition and virtually one hundred per cent mortality. Variation in the intake of vitamins and in other dietary constituents exerted little *or no protective effect. Slower growing animals in the same colony,* fed a so-called natural diet did not show any evidence of the disease. Further studies, however, indicated that the protective effect of the natural diet was correlated with the presence of certain foodstuffs such as alfalfa meal (209). Additional research is necessary to determine the stress effect of growth upon resistance especially since internal growth is correlated with a so-called balanced complete diet.

GROWTH AND NITROGEN BALANCE

Emphasis should be placed upon the maintenance of a positive *nitrogen balance for growth. Indeed the mechanism for growth* is so dominant that it may be difficult to produce negative balance in a growing child, at least over a short period of time, when the child is fed an adequate nitrogen intake even though the pattern of amino acids is markedly deficient. In any experiment involving correlation between nitrogen balance and growth, consideration should be given to the direction the balance is taking with time. An *ideal* pattern of amino acids for growth will maintain a positive balance for tissue synthesis and maintenance of this balance is one of the best criteria for the efficiency of a dietary protein. Nitrogen balance,

however, is a very sensitive indicator of changes in physiological state. The excretion of urinary nitrogen often increases and nitrogen balance becomes more negative in the presence of fever, infections, or injuries. It is possible that this increased utilization of the protein reserves in the presence of stress is part of a recovery or homeostatic mechanism.

PROTEIN AND MAINTENANCE

The significance of maintenance was developed, using the concepts of "protein reserves" in the discussion on "Nitrogen Balance." These concepts will now be considered further to lay the foundation for correlation between the determination of nutritive value of dietary proteins and nitrogen balance.

URINARY NITROGEN AND MAINTENANCE

The previous discussion of the dynamic state of the body suggested that some of the amino acids of the integrated pools enter into catabolic pathways leading to oxidative deamination and the formation of urea which is excreted as a waste product in the urine. Thus urea can arise from breakdown of protein reserves or from dietary amino acids. Data were presented earlier to indicate that the excretion of endogenous urea increases and decreases with the size of the metabolic pool so that, in general, urea excretion is high when the reserves are maximum and low when they are depleted. The excretion of urea of exogenous origin, on the other hand, varies with the efficiency of utilization of the dietary protein, being high when the pattern of amino acids is poor for protein anabolism or when the dietary protein is in excess of the needs of the body.

Another major loss of nitrogen from the body, discussed earlier, is represented by creatinine which is formed through muscular metabolism and is not directly affected by the magnitude of the amino acid pools of the body. Indeed the excretion of creatinine, which in young active adults can be approximately 21 to 25 mg. per day per kg. of body weight, is used as a measure of the magnitude of the muscle mass or of the accuracy of the daily collection of urine. Under ordinary normal conditions, the excretion should be constant and independent of the diet for any one individual. Standard, Wills, and Waterlow (210) used the excretion of creatinine as a measure of muscle mass in malnourished infants. They

found that, after two months of repletion on a milk diet, the excretion of creatinine was more than doubled. This increase in creatinine was of a relatively greater order of magnitude than the gain in body weight, which supports the belief that body weight deficit underestimates the degree of protein depletion (211).

Although amino acids are normally excreted in small amounts in the urine, the amounts and pattern may have some clinical significance (212-214). Cheung *et al.* (214) suggested that an abnormal pattern of amino acids may be excreted by patients with kwashiorkor. For example, high ratios of excretion of phenylalanine to tyrosine and isoleucine to leucine were observed in three patients. Schendel *et al.* (212) found an aminoaciduria to be associated with kwashiorkor, a condition which was corrected upon repletion. This aminoaciduria, however, was the result of an increased output of 17 amino acids. An increase in excretion of amino acids was also observed in both normal infants and those with kwashiorkor when they were fed a synthetic amino acid formula, the excretion being higher in those with maluntrition than in controls. Part of this increase was attributed to the DL isomers used in the synthetic mixture although overloading the metabolic pool with normal isomers will also increase the excretion of alpha amino nitrogen.

An increased excretion of urinary nitrogen is often associated with disease and with injury. Such an increase could be the result of a number of biochemical changes such as a shift in endocrine balance, the hypoxia of reduced blood flow, or the break down products of injured tissue. Cuthbertson (215, 216) who has studied this problem extensively suggested that under certain circumstances the labile tissue proteins which are catabolized following injury may provide energy and amino acids for the healing process. Glycogen and fat stores would be involved as well as the proteins in providing the energy and the metabolites for the healing process during a period when food intake might be quite limited. As Levenson and Watkin (217) have pointed out, it may not be necessary in many cases to overcome this excessive early loss of urinary nitrogen. The first problem may be to help the body utilize the reserves for recovery by furnishing fluids, reducing hypoxia, correcting electrolyte or endocrine imbalance to favor anabolic and

recovery processes. Repleting protein reserves, however, may very well be a most important feature in the recovery process, and in preventive medicine.

NITROGEN BALANCE INDEX FOR MAINTENANCE

Nitrogen balance will measure the over-all loss or gain in body nitrogen for maintenance purposes. In general, an individual eating an average American diet will have a relatively high protein intake and adequate reserves with high catabolic and anabolic activity. Such an individual would need a relatively high protein intake to maintain this activity. Conversely, depletion in these reserves could lower catabolism, reduce the excretion of urea nitrogen and thereby reduce the dietary protein requirement. Thus nitrogen equilibrium can be established on relatively high or low nitrogen intakes. The establishment of equilibrium, therefore, is not a direct measure of the adequacy of protein intake, but is a dramatic example of the ability of the body to adjust and to adapt to changing dietary environment and maintain homeostasis.

It is possible, however, to control balance experiments in man and in animals so that quantitative measurements can be made of the nutritive value of dietary proteins. If the physiological state of the individual is kept constant without significant changes in reserves the relationship between nitrogen balance and nitrogen intake is illustrated by the curves plotted in Figure 6 (72, 169, 218-221). The curves in this Figure were synthesized by assuming a nitrogen excretion of 5 grams per day at zero nitrogen intake, an excretion that represents nitrogen of endogenous origin. The position of the lines were calculated from nutritive values such as those determined by Hawley *et al.* (220). Curve A refers to egg protein; B, to casein; C, to peanut flour; and D, to wheat gluten.

These curves could be described as expressing the "law of diminishing returns" (222), reaching a maximum or optimum intake for filling the protein reserves for a given physiological state. This maximum varies with the nutritive value of the dietary protein. Emphasis is placed upon the physiological state because the curves are generally shifted up the ordinate, becoming more and more positive as the protein reserves are depleted. Such a shift to-

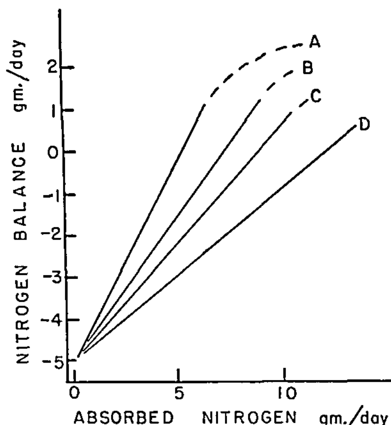


Figure 6. Nitrogen balances estimated for adult man receiving different intakes of egg protein A; casein B; peanut flour C; wheat gluten D.

ward positive balance demonstrates the reduction in the amount of nitrogen needed to maintain equilibrium and the greater potential for growth in nitrogen by the depleted individual. Thus the greater the depletion the more positive the nitrogen balance. Theoretically the nitrogen balance could never be positive in an adult if the protein reserves were filled.

The tangent to the curve at any absorbed nitrogen value has been called the nitrogen balance index for that nitrogen intake. The curves are essentially linear in the region of negative balance so that the indexes are constant in this region and at the point of nitrogen equilibrium. The constant slope extends into positive

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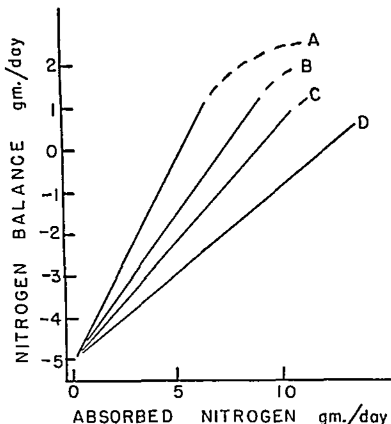


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balance for some distance if protein reserves can be repleted. The index is zero when the curve reaches maximum positive balance. This maximum can vary from a point on nitrogen equilibrium to one well in positive balance for animals with a potential for growth in body tissue proteins. Such maxima can be used to describe the needs for nitrogen by the individual as well as the nutritive value of the protein. These curves apply equally well for growth and maintenance in the infant and child as for repletion and maintenance in the adult. Thus maximum balances can be used to determine the requirements for both growth and maintenance.

Since the slopes of the curves in the region of negative and low positive balances are essentially linear, the index over this region is constant and can be used as a measure of the nutritive value of the dietary protein and may be calculated from the following equation:

$$\text{Nitrogen Balance Index} = \frac{B - Bo}{A} \qquad (V)$$

where B is the nitrogen balance produced by absorbed nitrogen A and Bo is the balance produced at zero nitrogen intake. The slope for curve A is 0.94; for B, 0.7; for C, 0.56; and for D, 0.42.

The value Bo, of course, represents the excretion of body nitrogen. If this excretion is constant and independent of nitrogen intake then the slope of those lines represent the fraction of absorbed dietary nitrogen retained in the body for tissue synthesis. This fraction was defined by Thomas (223) as the "Biological Value" of the dietary protein, a concept which was developed so well by Mitchell (35, 224). According to this concept, ninety-four per cent of the egg protein nitrogen was retained in the body for tissue protein synthesis whereas only 42 per cent of the wheat gluten nitrogen could be utilized for this purpose. In general, the assumption that the slopes of the lines as illustrated in Figure 6 are equal to "Biological Values" has been well established by Mitchell (35) and others (196, 225). There are situations, however, where the nitrogen excretion originating from the body changes with nitrogen intake so that Bo does not represent a constant excretion of body nitrogen and the slopes of the lines in negative balance can become

greater than unity (226). Under some experimental conditions the lines become curvilinear in the region of negative balance (138). For these reasons the slopes of the curves in Figure 6 will be called nitrogen balance indexes with the understanding that they have essentially the same significance as "Biological Value."

ADAPTATION TO NITROGEN EQUILIBRIUM

Adaptation of the body to produce nitrogen equilibrium is illustrated by the data recorded in Figure 7. These data were obtained while feeding dogs a protein-free diet (white bars) followed by the *same intake of the diet to which wheat gluten had been added* (bars with slanted lines). The long continued periods of negative balance reduced the excretion of body nitrogen and established equilibrium in animals fed the relatively low intake of wheat gluten nitrogen.

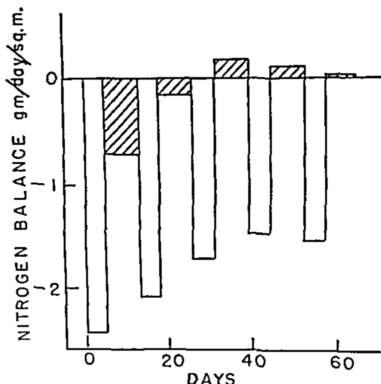


Figure 7. Nitrogen balances in dogs fed a protein-free diet (white bars) alternated with wheat gluten protein (bars with slanted lines).

Adaptation in this experiment was quite regular because of the controlled conditions. Great variations, however, in balance can occur with marked shifts in diet and in the physiological state. An adjustment always accompanies a shift from one diet with its characteristic metabolic pool to another. In general, the direction the balance takes is just as revealing, sometimes more so than the magnitude of change. A drift toward negative balance can be the result of a deficient diet or the result of some stress such as a fever or infection. Drugs or hormones affecting the activity of the endocrine system can increase or decrease endogenous metabolism (108).

Nitrogen balance, therefore, is a useful tool to determine dietary protein requirement but since it is an expression of sums and losses of nitrogen from all metabolic pools of the body and since it is very sensitive to shifts both in diet and physiological state the interpretation of changes in nitrogen balance requires careful consideration of these many factors.

REPLETION OF DEPLETED RESERVES

In the above discussion, emphasis was placed upon the potential for repletion of protein reserves in the adult, a potential that could be estimated in terms of the maximum positive nitrogen balance that could be produced. Data indicate that the amino acids needed for repletion of depleted adult tissues are essentially the same as those needed for growth. The pattern of amino acids needed for repletion of depleted rats, for example, was studied by Cannon and his associates (227). They found that an adult rat could be depleted and repleted in body tissue proteins by altering the amount and kind of protein in the diet and that this depletion and repletion could be accurately determined simply by measuring the loss and gain in body weight. The nutritive values of dietary proteins and amino acid mixtures, determined in this way were very similar to those reported from studies on growth in rats.

Repletion of depleted adult tissues can be illustrated also by the data recorded in Figure 8. These data were obtained by feeding adult dogs a protein-free diet for a period of four weeks (228). Wheat gluten or casein was added to the diet during repletion so that they received a constant intake of calories and nitrogen until

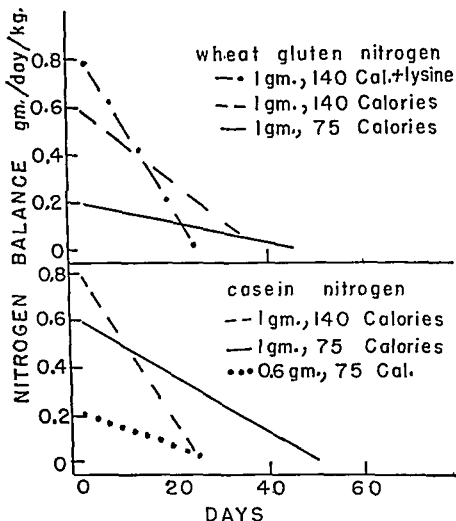


Figure 8. Repletion curves for dog fed casein or wheat gluten nitrogen with various caloric intakes (228).

equilibruim had been closely approached. Consider the upper part of Figure 8 as an example. The solid line in this figure illustrates the type of data obtained when the dogs were fed 1 gm of wheat gluten nitrogen and seventy-five calories per day per kg of body weight during the repletion period. The slope of this line is defined here as repletion index and is equal to 0.0046. The area under the

curve represents the amount of wheat gluten nitrogen retained in the body and will be called the area of repletion. Continued feeding of this diet would produce a small positive balance, thereby gradually filling the protein stores more adequately but requiring a long period of time. The caloric intake of seventy-five calories per day per kg of body weight is the average requirement for these adult dogs with normal protein reserves and living under laboratory conditions. Increasing the caloric intake from seventy-five to one-hundred-forty calories per day per kg of body weight resulted in data illustrated by the dashed line in the wheat gluten portion of the Figure. The slope of this line is 0.017 and the amount of nitrogen added to the body would be 10 grams, which is close to the body needs for repletion. Adding lysine to the wheat gluten to correct in part for the deficiency in the amino acid pattern resulted in data illustrated by the dash-dot line. The slope of this line is 0.032 and the area of repletion is also 10 gms. of nitrogen. Thus adding lysine to the wheat gluten increased the rate of repletion markedly.

The data obtained while feeding casein are illustrated in the lower part of Figure 8. The solid line illustrates the rate and amount of repletion obtained while feeding 1 gm of casein nitrogen at the lower caloric intake of seventy-five calories per day per kg of body weight. The slope of this line is 0.012 and the amount of repletion is represented by 15 gms of nitrogen. Increasing the intake to one-hundred-forty calories per day per kg of body weight raised the rate of repletion (slope of the line) to 0.032 but the amount of repletion reached approximately at nitrogen equilibrium was 10 gms of nitrogen, less than the 15 grams added to the body over a longer period of time at the lower caloric intake. In general, rapid repletion at a high caloric intake illustrated by the dashed line in this curve seems to represent a good way to replete the depleted reserves of the body. Continued feeding of the high intake, however, produced an obese animal whereas feeding the lower caloric intake prevented development of obesity. The dotted line in this Figure illustrates the rate and amount of repletion when the nitrogen intake was reduced to 0.6 gm per day per kg. body weight with an intake of seventy-four calories. The slope of this line is 0.012, the same as the slope at an intake of 1 gm of nitrogen per day per kg of

body weight with seventy-five calories. The amount of repletion represented by the dotted line, however, is only 2.5 gms of nitrogen. Thus repletion may be said to be correlated with three dimensions, nitrogen intake, caloric intake, and the pattern of amino acids provided by the dietary protein.

CALORIES AND PROTEINS

THE DATA PRESENTED previously indicate that there is an optimum level of caloric intake required to develop and to maintain an efficient balance between fat stores and lean body mass. For example, a weanling Beagle puppy required 1 gram of nitrogen from protein of high nutritive value per day per kg of body weight for growth and maintenance. With this nitrogen intake the puppy also needed approximately 200 calories per day per kg of body weight (230). These requirements decreased to 0.5 gm of nitrogen and 125 calories at ten weeks of age, and finally reached approximately 0.3 gm of nitrogen and seventy calories per day per kg of body weight during adulthood. Possibly the protein requirement should be expressed in terms of calories. For example, feeding protein of high nutritive value and an optimum caloric intake, approximately 10-12 per cent of the total calories should be protein calories, for growth, repletion or maintenance.

The interrelationship between caloric intake and nitrogen metabolism has been presented recently in an excellent review by Munro (231). No attempt will be made to present such a review here except to emphasize again the evidence that the body utilizes protein reserves to correct a deficiency in calories in the metabolic pool and, conversely that carbohydrate and fat calories conserve body nitrogen (228). The decrease in nitrogen balance and increase in excretion of urea nitrogen is illustrated in Figure 9. These data were obtained by feeding different caloric intakes but with a constant dietary protein intake to dogs (207). Obviously, if the caloric intake is reduced below a critical value, dietary proteins of high or low nutritive value will be utilized for energy purposes, the retention of nitrogen being reduced to a minimum.

Some of these effects of caloric intake in the presence and absence of dietary protein are illustrated by the data in Table II. Groups of adult rats were fed different caloric intakes over a period of thirty-six days. Some received 250 mg. of casein nitrogen per

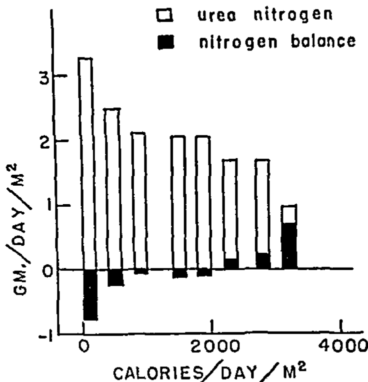


Figure 9. Urea nitrogen (white bars) and nitrogen balance in dogs fed a constant nitrogen but a variable caloric intake. Published with permission of *Am. J. Clin. Nutrition* (207).

day. Others were fed the diet without the addition of the casein. The data demonstrated that animals fed seventy-four calories per day per rat, together with the 250 mg of casein nitrogen per day, gained an average of 109 grams in weight over the experimental period of thirty-six days. Those fed forty-three calories per day with the casein in the diet just maintained body weight while those fed the casein diet and eighteen calories lost an average of 110 grams in weight. The animals fed the protein-free diet lost approximately 70 grams regardless of the caloric intake (229).

The total liver weight as well as the liver protein decreased in a regular way with decreased caloric intake in the animals fed casein. A reduction in calories reduced liver protein even though the dietary protein intake was constant. The data indicate that

TABLE II

TISSUE COMPOSITION OF RATS FED 250 MG. OF CASEIN NITROGEN PER DAY OR A PROTEIN-FREE DIET AT DIFFERENT CALORIC INTAKES OVER A PERIOD OF THIRTY-SIX DAYS. THE AVERAGE INITIAL BODY WEIGHT WAS APPROXIMATELY 350 GRAMS (228, 229).

Diet	*Casein*	*Protein-free*	*Casein*	*Protein-free*	*Casein*	*Protein-free*
Caloric intake per rat per day	74	76	43	46	18	19
Body weight	459	273	355	289	242	282
Liver weight g.	12.2	10.0	9.3	9.1	6.4	7.5
Lipid % dry weight	32.6	56.9	28.2	45.7	28.6	28.9
Protein g.	2.3	1.1	1.9	1.2	1.3	1.2
Heart weight g.	1.1	0.7	0.9	0.8	0 7	0.8
Protein g.	0.16	0.11	0.14	0.12	0.11	0.13
Kidney weight g.	2 6	1.7	2.3	2.2	1.9	2 0
Protein g.	0.39	0.26	0.34	0.32	0.28	0.31

there was little dietary nitrogen retention in rats fed the casein diet at a level of eighteen calories per day. The liver protein in these animals was approximately equal to the liver protein in animals fed the protein-free diet. Thus the limiting caloric intake for nitrogen retention in these adult rats was close to eighteen calories per day. The effect of protein in the diet upon the fat in the liver is also illustrated in part by these data. At the low caloric intake (18-19 calories per day) the fat content of the liver was approximately 29 per cent expressed on a dry weight basis whether protein was in the diet or not. However, when the caloric intake of the rat was increased to 76 calories per day the fat content of the liver increased to 57 per cent in animals fed the protein-free diet while it remained at approximately 33 per cent in animals fed this same caloric intake but in the presence of casein.

The data in Table II also illustrate the loss in weight of the heart and kidney, associated with a reduction in caloric intake even though the protein intake was not reduced. The protein of the heart and kidney were also reduced with caloric intake. These organs, however, did not lose weight as rapidly as the body as a whole so that there was some conservation of them in the depleted animal. The data involving the heart and kidney again indicate that 18 calories per day resulted in little or no nitrogen retention.

Under some circumstances the effect of carbohydrate, fat and amino acids on nitrogen retention during caloric restriction can vary (231-234). Van Itallie (234) pointed out that calories from carbohydrate, fat and amino acids should not always be placed together under the label of fuel. With the reviews of Calloway and Spector (235, 236) as a background he suggested, however, that an adult male would lose approximately 12 gm of nitrogen per day early in starvation. This loss could be reduced to 7 gm per day when the intake was raised from 0 to 700 calories from non-protein sources. The data suggest that little or no nitrogen is retained when the total dietary intake is as low as 400 to 600 calories. Schwimmer and McGavack (237) found that a reduction in caloric intake to approximately 25 per cent of normal resulted in little or no retention of dietary nitrogen in adult young men. This limiting intake for nitrogen retention, however, could vary with the physiological state of the individual and with the nitrogen intake.

Thus the initial response to reduced calories is increased excretion of urea nitrogen without altering the nitrogen balance index of the dietary protein. The data indicate that, within physiological limits, the body adapts to a caloric restriction although there is a limiting energy level for protein synthesis.

NUTRITIVE VALUE AND PROBLEMS IN SUPPLEMENTATION

THE DETERMINATION of nutritive values through the use of nitrogen-growth indexes, protein efficiencies, nitrogen balance indexes, and repletion-indexes have been described. It is common practice to determine the nutritive value of single dietary protein sources which can be illustrated by the nitrogen balance index data recorded in Table III. These data were obtained in different laboratories but using the same samples of protein (238). The indexes are remarkably similar for man, dog, and the rat, except for some outstanding exceptions. The index, for example, of wheat gluten protein fed to the normal dog was 0.40 but it was 0.70 in the protein-depleted animal, a result which can be interpreted to mean that the efficiency of utilization of a pattern of amino acids is increased in the protein-depleted individual (239). The index was 0.65 in the adult rat for maintenance but was reduced to 0.40 for growth, a difference which was interpreted to mean that the amount of dietary lysine was not as critical for maintenance as for growth in this animal (240).

TABLE III
NITROGEN BALANCE INDEXES FOR ABSORBED NITROGEN
(TAKEN FROM REFERENCE 26)

Subject	*Whole Egg*	*Egg White*	*Beef*	*Casein*	*Peanut Flour*	*Wheat Gluten*
Man (adult)	0.94	0.91	0 67	0 68	0.56	0.42
Dog (normal adult)	0.87	1.14	0.77	0.73	0.56	0.44
Dog (depleted adult)	1.06	1.14	....	0 84	....	0.70
Rat (adult)	0 82	0.94	0.69	0 51	0 46	0 65
Rat (growing)	0 87	0 97	0.76	0 69	0.54	0.40

Few ordinary diets, however, are made up of single dietary proteins so that the major interest is centered around the ability of one protein to improve the nutritive value of another. For example,

DeMaeyer and Vanderborght (72) recently published a study of the nutritive value of various dietary sources of proteins in the feeding of African children. The children varied in age from three to seven years. They were fed a mixed diet containing rice, banana flour, bread, fresh banana, red palm oil, butter, sugar, orange or lime and a vitamin and mineral mixture. These authors found an excellent linear correlation between nitrogen intake and positive nitrogen balance so that indexes could be accurately determined. They reported that milk had the highest supplementary value with a combination of beans and peanuts next in value. These authors also found that in severely protein-depleted children the nitrogen retention was markedly increased, a result reported also for animals (239). Deficiencies can also be corrected by amino acid supplementation (241).

Simply improving the index by supplementation, however, without adjusting the protein intake to meet requirements for growth or maintenance would not be nutritionally adequate. Adding a meat sauce, for example, to spaghetti improved the nitrogen growth index for rats markedly so that the nutritive value of the protein of the mixed diet, which is designated as A, was relatively high. The amount of protein in the mixed diet A, however, was low relative to caloric intake so that the animals could not grow normally when fed this supplemented food. Another alimentary paste with a higher protein content and a higher nutritive value was also improved by the meat sauce although the index of this mixed diet, which is designated as B, was not any higher than A. Diet B, however, provided the proper ratio between calories and protein so that the rats grew normally when they were fed this higher protein intake (161).

Thus deficient patterns of various foods can be corrected in whole or in part by mutual supplementation, a corrective process that can be adequate in the average American mixed diet, particularly because of an abundant supply of animal protein. Many diets, however, in the technologically under-developed parts of the world are based on food sources which have a low protein content with a deficient pattern of amino acids. People eating these diets have

learned in part to supplement the deficient plant food. Beans, for example, are often added to a corn diet which increases the protein content and improves the pattern so that adults may exist on these partially supplemented diets. If, however, there is a deficiency in pattern and intake, the results of protein malnutrition will be most evident in the children (63). The protein reserves are minimum and soon depleted in these children, especially when the diet is restricted during an illness. Furthermore, the children often are given diets much more deficient in protein than the adults, a diet consisting largely of gruels. A dietary survey, therefore, of a population may not reveal accurately the deficiency that is developing in a particular age group or in individuals.

It would seem best to develop an agriculture in many of these countries that would provide a mixture of plant proteins, with a relatively high nutritive value for both animals and man. Such a source for plant proteins would provide an economical basic vegetable diet for man and still allow him to produce sufficient animal products to ensure good supplementation not only of amino acids but also of other essential factors which are associated with foods such as milk, eggs, and meat. Indeed the development of an animal industry is possible and essential to the proper growth of agriculture in most of these countries (242). Such a program as this has been started by INCAP in cooperation with agricultural agencies of member countries (89). A vegetable mixture, for example, of high nutritive value has been prepared that is economic and very acceptable as a dietary supplement for children and adults, a food that requires little or no readjustments in the eating habits of many families that are in need of increased protein intake. Some of the basic work in agriculture and animal nutrition leading to the development of the mixture was done by Squibb and associates (243) working in the Agropecuario Nacional, a former cooperative U. S. Department of Agricultural Station. The composition of the mixture, biochemistry, physiology, human nutrition, and medical applications were developed at INCAP, an example of the value and need for an interdisciplinary approach to the solution of these serious nutritional and health problems.

THE REFERENCE PATTERN

These discussions of nutritive values and problems of supplementation emphasize the need to provide an efficient pattern of amino acids for protein synthesis and to balance properly the amino acids with the energy requirements. Possibly a good general rule would be to recommend the improvement of the nutritive value of dietary proteins if the nitrogen balance index of a mixture of these proteins is less than 0.6. Since the efficiency of the pattern of amino acids is largely determined by the proportion of indispensable amino acids, various reference mixtures of these acids have been suggested to determine deficiencies or imbalances in the diet. The most common reference has been the pattern of essential amino acids in the egg proteins since these proteins have a high nutritive value (32, 34). Another reference can be established by analyzing the carcass of animals for amino acid patterns associated with maintenance and growth (244). The committee on Proteins of the Food and Agriculture Organization (FAO) of the United Nations, however, decided to develop a reference pattern from data obtained in studies where amino acids were used in the diet instead of protein (36). It was hoped that such an approach would reveal more directly the significance of a pattern of amino acids and would provide another check upon the concepts of the interrelationship between nutritive values and various mixtures of amino acids. The evidence presented in the literature suggests that amino acids are liberated very rapidly in the intestine from dietary proteins of high nutritive value and that, in general, dietary proteins can be supplemented adequately by adding free amino acids to overcome any deficiency (152, 153, 241). Thus a mixture of free amino acids may be absorbed and utilized in a similar way to a dietary protein which is rapidly and efficiently digested. A mixture of free amino acids fed to man, however, required a higher intake of calories to bring about maintenance of nitrogen equilibrium than would be required by an equivalent amount of dietary protein. It is possible that the rapid presentation of free amino acid for transportation and sequesteration and possibly a relatively high proportion of

certain dispensable amino acids in the diet could increase the demand for dietary calories. *However, this would not necessarily* alter the pattern for maximum nutritive value.

The FAO committee recognized that the reference pattern could vary with age and the physiological state of the individual but it was decided to start with a single reference derived from the nitrogen balance studies of Rose and associates (1-4) Leverton *et al.* (245-249). Swendseid *et al.* (250, 251) Clark, Mertz *et al.* (252, 253) and growth studies in babies such as those reported by Holt, Snyderman and associates (254-259). A review of the amino acid requirements of man has been published recently by Reynolds (258). Each amino acid in the reference pattern was expressed as a ratio to tryptophan, which is a customary and logical approach to establish chemical interrelationships with the amino acid in lowest concentration. Tryptophan, however, can enter several metabolic pathways other than protein synthesis as can a number of the other amino acids. For that reason the ratio has been expressed in terms of unity for threonine in Table IV. This does not mean that threonine is the best choice for such a series of ratios but it illustrates the basic similarity between the human milk and egg patterns and the others recorded in Table IV. The FAO reference pattern is very similar to the egg pattern except for tryptophan. The reason for the relatively high tryptophan requirement for man fed mixtures of amino acids is not clear but the data emphasize the importance of this approach to the solution of the pattern problem. The experiments of Howe *et al.* (151) demonstrated the need of having the correct percentage of indispensable amino acid pattern in the amino acid mixture or in the dietary protein to meet the requirements of the body for synthesis. The amount of synthesis was limited not only by a limiting indispensable amino acid but also by a limiting amount of the pattern of indispensable amino acids. As pointed out by Harper and Kumta (29), egg proteins contain approximately 60% while wheat gluten has only a bit over 40% of the indispensable or semi-indispensable amino acids, so that wheat gluten is not only deficient in lysine but also in total indispensable amino acids for maximum growth in the

THE REFERENCE PATTERN

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TABLE IV

INDISPENSABLE AMINO ACID PATTERNS FOR MAINTENANCE AND GROWTH WITH THREONINE TAKEN AS UNITY (161)

	Reference			*Maintenance*		*Growth*	
	Human Milk	*Egg*	*FAO*	*Man*	*Dog*	*Rat*	*Chicken*
Arginine	0.9	1.2	...	...	...	0 8	2.0
Histidine	0.5	0.5	...	...	0.5	0.5	0.5
Isoleucine	1.2	1.3	1.5	1.3	1.6	1.4	1.0
Leucine	2.0	1.8	1.7	1.8	2.3	1.9	2.3
Lysine	1.4	1.5	1.5	1.1	1.5	1.6	1.8
Phenylalanine	1.0	1.2	1.0	1.2	1 1	1.3	0.8
Tyrosine	1.1	0.6	1.0	0.7	0.8	0.7	0.8
Total Aromatic	2.1	1.8	2.0	1.9	1.9	2.0	1.6
Total Sulfur	0.9	1.5	1.5	0.8	1.4	1.4	1.4
Threonine	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tryptophan	0.36	0.24	0.5	0.24	0 27	0 21	0.25
Valine	1.4	1.5	1.5	1.5	1.5	1 7	1.30

rat. It is possible, however, that man may need a smaller percentage of indispensable amino acids than those found in milk or eggs (255).

The patterns for maintenance in adult man and in the adult dog were calculated from data (161, 230) where the minimum requirements for each amino acid were derived from nitrogen balance experiments involving a series of dietary proteins. It was assumed that sufficient dietary proteins were considered to estimate the limiting values of each indispensable amino acid, an assumption that was used also by Harte and Travers (261). The minimum intake of a protein which presented an ideal pattern of amino acids to the body would be approximately 0.4 gm per day per kg of body weight. This amount would maintain the very minimum protein reserves for the adult illustrated in Figure 2. These data in Table IV suggest that the requirement for lysine and for sulfur amino acids for maintenance in the adult man may be below the requirement illustrated by egg and FAO patterns. It should be emphasized, however, that, repletion of depleted protein reserves in the adult animal requires the same pattern as for growth in the young. A good general rule, therefore, is to estimate the requirements for the adult both from the viewpoint of pattern and percentage of energy requirements to be approximately the same as for the young. To obtain minimum requirement for maintenance in man expressed as mg. of amino acids per kg of body weight, multiply the values in Table IV by 19.5, for maintenance in the dog multiply by sixty-one.

The pattern for growth in the rat and the chicken are essentially the same as the egg pattern. The data for the rat were taken from a previous publication (161) and those for the chicken from papers by Fisher and associates (262-266). The rat needs less lysine for maintenance than for growth (240) but it is recommended that the same pattern be used for both young and old to take care of periods of repletion that follow starvation or illness in the adult. To calculate grams of amino acid needed for growth of 50 grams in the rat multiply by 0.44. Fisher has demonstrated that the pattern for maintenance in the chicken is affected by the demand for the growth of the feathers and egg production.

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	Reference			*Maintenance*		*Growth*	
	Human Milk	*Egg*	*FAO*	*Man*	*Dog*	*Rat*	*Chicken*
Arginine	0.9	1.2	...	...	...	0.8	2.0
Histidine	0.5	0.5	...	...	0.5	0.5	0.5
Isoleucine	1.2	1.3	1.5	1.3	1.6	1.4	1.0
Leucine	2.0	1.8	1.7	1.8	2.3	1.9	2.3
Lysine	1.4	1.5	1.5	1.1	1.5	1.6	1.8
Phenylalanine	1.0	1.2	1.0	1.2	1.1	1.3	0.8
Tyrosine	1.1	0.6	1.0	0.7	0.8	0.7	0.8
Total Aromatic	2.1	1.8	2.0	1.9	1.9	2.0	1.6
Total Sulfur	0.9	1.5	1.5	0.8	1.4	1.4	1.4
Threonine	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tryptophan	0.36	0.24	0.5	0.24	0.27	0.21	0.25
Valine	1.4	1.5	1.5	1.5	1.5	1.7	1.30

Recently the group at INCAP (89) have been testing the usefulness of the FAO pattern for the determination of amino acid requirements in growing children with or without protein malnutrition. They have used the nitrogen balance technique very successfully and have demonstrated that adding tryptophan and lysine to corn and by correcting the imbalance in the leucine, isoleucine ratio the nutritive value approaches that of milk. It was such studies as these that made possible the development of a vegetable mixture that would prevent or cure kwashiorkor. Their data not only emphasize the usefulness of the reference pattern but also point to the need for considering the rate of digestion of various vegetables proteins as a factor influencing the actual pattern presented to the body. The chemical analysis for amino acids may not always give an accurate picture of the pattern made available to the body by a mixture of dietary proteins.

Lack of balance between amino acids can cause adverse effects. For example, if an excess of methionine is added to casein it will retard growth and decrease nitrogen balance, thereby increasing the loss of body nitrogen (127, 267, 268). This toxic effect can be overcome by adding glycine, possibly even better by adding glycine plus arginine with the excess methionine. The data indicate that the glycine and arginine contribute to the formation of guanidinoacetic acid which is methylated by the methionine to form creatine, thus supplying reagents to utilize the excess methionine and reduce its toxicity. Indeed guanidinoacetic acid will reduce the toxicity of excess methionine and conversely methionine will reduce the toxicity of excess guanidinoacetic acid. The suggestion was made previously that such so called "imbalances" may be used to direct certain chemical changes in the body, possibly some of them having therapeutic usefulness. Certainly more work needs to be done on this suggestion. The terms "imbalance" and "unbalance" have been used interchangeably in the literature to refer to situations that are not always comparable. Harper and Kumta (29) have defined "unbalance" as a protein low in one or more of the indispensable amino acids. An "imbalance" is the situation which develops upon the addition of an unbalanced protein to cause some adverse effect which can be prevented by supplementation. The

importance of giving careful consideration to nitrogen intake and amino acid balance in the diet of man was revealed also by the studies of Hundley *et al.* (269).

The amino acid composition of the blood, as an integral part of the metabolic pool, deserves considerable study. Longenecker and Hause (140), for example, have reported some excellent correlations between nutritive value of a dietary protein and the pattern of amino acid in the blood. They assumed that the individual indispensable amino acids were removed from the blood by the tissues of the body at a rate which was proportional to the requirement. They found that the changes in concentration of amino acids in the blood following a meal revealed the nutritional adequacy of the indispensable amino acid pattern provided by the diet.

PLASMA AND LIVER PROTEINS

The body proteins that have been studied most extensively in relationship to protein malnutrition are those found in the liver and serum or plasma. The loss of liver protein during depletion in protein reserves has been discussed previously, this loss being often described as a reduction in activity of certain enzymes systems (47-55, 270). The imbalance in the activity of liver enzymes in the depleted animal is illustrated in Figure 10, an imbalance which is not so marked in the heart as in other tissues. As pointed out by

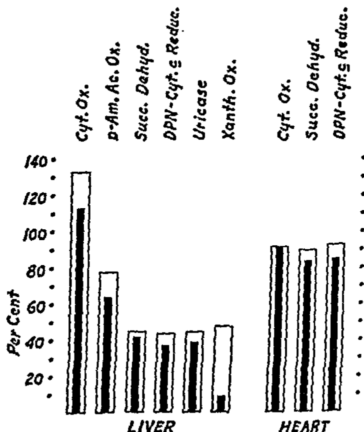


Figure 10. Unit enzyme activity (per mg nitrogen) in protein - depleted rats expressed as per cent of pair-fed controls (outer white bar) and as per cent of ad libitum fed controls (inner black bar). Cyt. Ox = cytochrome oxidase; Succ. Dehyd. = succinic dehydrogenase; D-Am. Ox = D-amino acid oxidase; DPN-Cyt. c Reduc. = DPN-cytochrome c reductase; Xanth. Ox. = xanthine oxidase. Published with permission of J. Nutrition (49).

Burch *et al.* (54), Waterlow *et al.* (55, 270), data obtained on malnourished children demonstrate that there are differences between normal and depleted livers in distribution and activity of enzymes. Waterlow suggested that future studies should lead to an understanding of the more complex functions which are effected by these differences.

If the caloric intake is relatively high, depletion in liver proteins is associated with the development of fat in the liver. Thus a fatty liver is commonly associated with kwashiorkor but not with marasmus. Nutritional factors can be important in the development of a fatty liver-cirrhosis syndrome (271-273). György (274) has written an excellent review on the effects of protein nutrition on the liver. As he pointed out, the liver is the central organ in metabolism, and therefore pathological alterations are intimately connected with protein malnutrition. In addition, there is an interrelation between dietary, endocrine, and genetic factors in the pathogenesis of hepatic injury. Preventive and therapeutic measures are not always interchangeable. Protein or lipotropic substances would be beneficial to prevent progression of a dietary cirrhosis but if the cirrhosis is severe, dietary treatment often fails. Certainly dietary protein would be important in the prophylatic and therapeutic measures connected with pathology of the liver. In acute severe hepatic injury, however, use of protein or methionine may not be beneficial. Further knowledge concerning the interrelation between the activity of enzyme systems in the injured or depleted liver and complex functions of the liver is needed (275).

The proteins of the blood have been studied extensively in relation to protein malnutrition and other diseases. The rise and fall in serum albumin with the increase or decrease in protein reserves was discussed under "protein reserves." The reduction in concentration of plasma albumin with the loss in body nitrogen in dogs fed a protein-free diet is illustrated in Figure 11 (276). The black circles in this figure record serum albumin concentration in dogs fed a protein-free diet containing 21% of a fairly highly saturated fat. The black triangles illustrate similar data obtained while feeding the diet containing the same quantity of a more unsaturated fat (corn oil). The kind of fat did not influence the rate

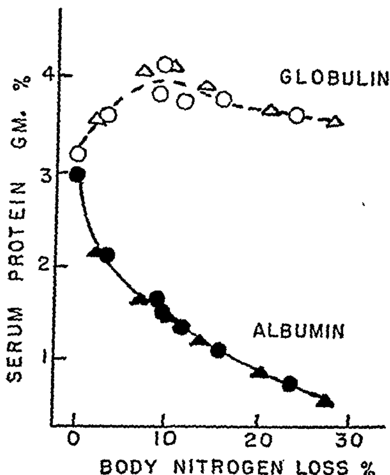


Figure 11. Serum albumin and globulins correlated with loss in body nitrogen in dogs fed a protein-free diet (276).

of depletion of serum albumin but it did influence the magnitude of the rise in serum lipids characteristic in protein-depleted dogs fed this high fat diet (277).

Previous studies have demonstrated a rise in plasma lipids with depletion in body nitrogen in dogs fed a relatively high fat diet (276). Low protein diets have produced elevated plasma cholesterol in other animals (278). The data suggested that this increase in plasma lipids could be the result of a biochemical overloading of depleted enzyme systems such as those systems involved in the oxidation and desaturation of fatty acids. The more saturated fat

in the diet resulted in a more marked rise in serum phospholipids, cholesterol esters and the lipids migrating with Alpha and Beta globulins. Repletion with casein resulted in the return of all values toward normal as illustrated for cholesterol in Figure 12 (276). On the other hand, repletion of the depleted adult with a relatively high intake of wheat gluten did not correct as rapidly this biochemical imbalance between serum proteins and lipids (276). In *the normal repleted dog, however, the type of protein or fat in the* diet did not change the plasma lipids from so-called normal values

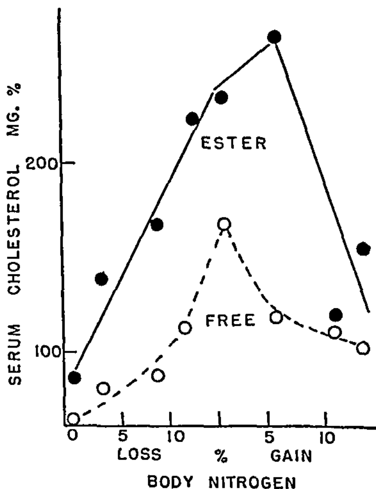


Figure 12. The increase and decrease in serum cholesterol with rise and fall in body nitrogen in dogs fed a relatively highly saturated fat diet (276).

even when they were fed in an obesity producing diet over a relatively short period of approximately one month. These results emphasize the ability of the body with normal reserves to maintain a certain biochemical balance in these serum constituents.

Serum lipids have been reported to be low in children suffering from protein malnutrition (279-282). Upon repletion, however, the lipids increased initially and then decreased toward so-called normal values. The low serum lipids in the protein-depleted individual could be the result of a diet low in fat and in other metabolites. The initial rise associated with repletion could represent an overloading of depleted enzyme systems resulting in a rise in lipids similar to those observed in the depleted animals. Serum lipids also may increase in animals suffering from nutritionally malignant tumors (described under "abnormal growth") a rise that has been associated with the depleting mechanism of the tumor. It was suggested that this mechanism might be correlated with reduced protein synthesis in normal tissues, possibly revealed by an increase in the ribonuclease activity. This increase in activity was observed also in the protein-depleted animals. An increase in plasma lipids was observed in rats with one kidney removed and a hypertension produced by the administration of deoxycorticosterone acetate (DCA). Thus imbalances in the plasma protein: plasma lipid ratios were associated with the physiological state of the animal as well as with the diet, a physiological state which may represent a reduction in protein synthesis and an overloading of certain enzymes systems (208, 276).

The excellent correlation between loss in body nitrogen and the reduction in concentration of albumin is not always found in depleted animals. An animal may be depleted in protein reserves, for example, and yet not have a decrease in the concentration of serum albumin and the total serum protein may be higher than normal (141). Such a condition has been observed in animals where the plasma volume has dropped so low that the concentration of proteins are high even though the total circulating albumin may be markedly reduced. Under these conditions the albumin: globulin ratio is reduced, and can be used as evidence of protein malnutrition. Associated with the fall in concentration of plasma

albumin illustrated in Figure 11, is a rise followed by a decrease in the total serum globulins. The concentrations of globulins, however, are seldom reduced below so-called normal values under these experimental conditions. Increased extracellular fluid was always found associated with depletion in reserves even though a clinical edema could not be observed. It is interesting to note that a high total serum protein with a relatively low serum albumin was found by Sebrell *et al.* (86) in malnourished Haitian peasants.

Other labile reserves in the serum, are revealed by a reduction in activity of various enzymes such as aldolase (37) and pseudocholinesterase (283) in depleted individuals. Sometimes, however, the magnitude of depletion, must be quite great, before the activity of the enzyme system is significantly altered from so-called normal variation. Arroyave *et al.* (284) for example, found that serum cholinesterase was not a sensitive measure of protein deficiency in children. The chemistry of the serum proteins also may be altered by depletion. The polarographic activity of the plasma proteins increased in individuals suffering from protein malnutrition (26), cancer, or pneumonia (59). This rise in activity has been interpreted to be the result of an increase in the number of free sulfhydryl groups. Possibly a reduced binding may explain the increased fragility of plasma protein to precipitation with certain chemical agents when the proteins are in plasma with this high polarographic activity. The filtrate, for example, from such proteins precipitated with sulfosalicylic acid contains relatively large amounts of unprecipitated polypeptides not present in the filtrate of normal serum.

One of the first experiments to demonstrate that dietary proteins affected repletion in plasma proteins was reported by Kerr, Hurwitz, and Whipple in 1918 (285). Many modern concepts of the dynamic state of body proteins, of the raiding of one tissue by another, originated or developed from the work of Whipple, Madden, Robscheit-Robbins, Miller, and associates (10, 285-287). Their studies emphasized the specific effect of different mixtures of amino acids or dietary proteins upon response, some mixtures favoring the formation of hemoglobin, others the various plasma proteins. In this connection dietary casein is an excellent protein

TABLE V

SERUM PROTEINS (GRAMS PER 100 ML OF SERUM) DETERMINED IN NORMAL, TUMOR-BEARING AND PROTEIN-DEPLETED DOGS. THE CORRESPONDING SPECIFIC ACTIVITIES (S.A.) (μG. S^{35} PER GM. PROTEIN) FOLLOWING INJECTION OF L-METHIONINE S^{35} (38).

Serum Protein Fractions

	Alb.	S.A.	*Alpha* 1+2	S.A.	*Alpha* 3	S.A.	*Beta* ...	S.A.	*Gamma*	S.A.
Normal	2.26	0.16	0.72	0.22	0.80	0.23	1.93	0.29	0.58	0.18
Cancerous	2.09	0.19	0.68	0.30	0.75	0.34	2.12	0.63	0.43	0.19
Depleted	1.12	0.28	0 71	0.26	0.69	0.28	1.96	0.70	0.72	0.28

source for the repletion of tissue proteins but it does not cause a rise in plasma albumin as rapidly as does lactalbumin (288, 289) beef (290) or wheat gluten (37) in depleted animals. Chow *et al.* (288), have presented data which indicate the existence of a fraction in hydrolysates of casein that promoted the regeneration of plasma globulins in animals fed lactalbumin hydrolysates. On the other hand casein supplemented with methionine or still better with methionine plus guanidinoacetic acid promoted repletion of plasma albumin in protein depleted dogs (37).

An increased uptake of S^{35} from labeled L methionine was reported in animals and children with protein malnutrition (39) or in animals with tumors (38). This increased uptake is centered primarily in the Beta-globulin fraction of the plasma from protein-depleted or tumor-bearing dogs, as illustrated in Table V. Such an increase may be associated with a reduced metabolic pool of amino acids and the conservation of certain body proteins in the presence of a depleting or conserving mechanism. Such mechanisms may be associated with a shift in ribonuclease activity previously discussed. Indeed these and other observations open the way to a more detailed study of changes in metabolism associated with depletion in reserves and the attempt of the body to protect itself from a severe loss of reserves and to maintain specific proteins of importance to the welfare of the living system. The significance of dietary proteins in health and disease may center, therefore, around the importance of these protein reserves in homeostasis and in resistance to stresses.

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INDEX